RESEARCH ARTICLE

Effect of Lymphangiogenesis and Lymphovascular Invasion on the Survival Pattern of Breast Cancer Patients

Pradyumna Kumar Sahoo[1], Debarshi Jana[2]*, Palash Kumar Mandal[3], Samindranath Basak[1]

Abstract

Background: Invasion of breast cancer cells into blood and lymphatic vessels is one of the most important steps for metastasis. In this study the prognostic relevance of lymphangiogenesis and lymphovascular invasion (LVI) in breast cancer patients was evaluated in terms of survival. Materials and Methods: This retrospective study concerned 518 breast cancer patients who were treated at Department of Surgical Oncology, Saroj Gupta Cancer Centre and Research Institute, Kolkata-700063, West Bengal, India, a reputed cancer centre and research institute of eastern India between January 2006 and December 2007. Results: The median overall survival and disease free survival of the patients were 60 months and 54 months respectively. As per Log-rank test, poor overall as well as disease free survival pattern was observed for LVI positive patients as compared with LVI negative patients ($p<0.01$). Also poor overall as well as disease free survival pattern was observed for perineural invasion (PNI) positive patients as compared to PNI negative patients ($p<0.01$). Conclusions: From this study it is evident that LVI and PNI are strongly associated with outcome in terms of disease free as well as overall survival in breast cancer patients. Thus LVI and PNI constitute potential targets for treatment of breast cancer patients. We advocate incorporating their status into breast cancer staging systems.

Keywords: Breast cancer - lymphovascular invasion - perineural invasion - survival

Asian Pac J Cancer Prev, **15 (15)**, 6287-6293

Introduction

Breast cancer (BC) is the most common cancer and the leading cause of deaths among women worldwide (Jana et al., 2012). BC is now the most common cancer among women in most cities in India, and 2nd most common in the rural areas. BC accounts for 26.3% of all cancers in women in Kolkata which is the commonest cancer among women. The age-specific incidence rate is 26.1 per 100,000 women (Sen et al., 2002; Ferlay et al., 2008). BC has the moderate prognosis among all the female malignancies. Though BC has the better prognosis compared to other female malignancies, primarily due to the late stage at diagnosis of the cancer the overall 5-year survival becomes poor. Most of the BC patients receive suboptimal management due to logistics and poverty (Jana et al., 2012; Khokhar, 2012). In rural areas of the state of West Bengal a large number of BC patients have poor access to any specialized cancer treating centers (Das et al., 2012). The huge cost of antineoplastic drugs is one of the major constrains for the patients to continue with the treatments as decided by the oncologists. Lack of awareness leading to advanced stage of disease at diagnosis and non-compliance to treatment mostly due to poverty are responsible for the poor survival rates of BC patients in this region. A very few research works related to BC survivals have been communicated from this region, so far. Thus the present study was conducted with the retrospective analysis of the records of BC patients treated Department of Surgical Oncology, Saroj Gupta Cancer Centre and Research Institute, Kolkata-700063, West Bengal, India, at a reputed cancer centre and research institute of eastern India. Prognosis of BC can be depended on the risk of aggressiveness, faster death or recurrence and shortened disease-free survival (DFS) or overall survival (OS). DFS and OS are commonly used in BC prognosis. Prognosis of BC is depended on clinical factors, histopathological parameters and hormone receptors (Cooke et al., 2001; Laguens et al., 2006; KK Ma et al., 2012). The major cause of death or relapse from BC is lymph node involvement. Spread of lymph node metastasis is first step process (Donegan, 1997; Irianiwati Widodo et al., 2013). So the detection of lymph node metastasis represents an important prognostic factor of BC patients and predicts the choice of additional chemotherapy and radiotherapy after surgery of tumor (Tan et al., 2005). Breast tumor related lymphatic vessels

*[1]Department of Surgical Oncology, Saroj Gupta Cancer Centre and Research Institute, [2]Institute of Post Graduate Medical Education & Research (IPGME&R) and Seth Sukhlal Karnani Memorial Hospital (SSKM), Kolkata, [3]Department of Pathology, North Bengal Medical College, Sushrutan Agar, Darjeeling, West Bengal, India *For correspondence: debarsheejana@gmail.com, debarshijana@yahoo.in*

are considered as the major route of tumor metastasis to axillary lymph nodes (Nathanson et al., 2000; Samad Muhammadnejad etal., 2013). Lymphovascular Invasion (LVI) involves lymphangiogenesis in BC. LVI among BC patients' correlations with several clinicopathological factors are important to find out prognosis and treatment strategies (Irianiwati Widodo et al., 2013). Presence of neovascularization in breast carcinoma may be an independent predictor of metastatic disease either with lymph nodes involvement or at distant metastasis. Modulation of tumor angiogenesis may be novel therapeutic application (Noel et al., 1991). Perineural invasion (PNI) is poor prognostic factor for survival in BC (Karak et al., 2010). In our study the survival pattern of BC patients had been compared with their status of LVI and PNI.

Materials and Methods

The medical records of all BC patients who were treated with surgery, chemotherapy and radiotherapy at Department of Surgical Oncology, Saroj Gupta Cancer Centre and Research Institute, Kolkata-700063, West Bengal, India, a reputed cancer centre and research institute of eastern India between January 2006 and December 2007 were reviewed. A total number of 518 BC patients were treated during that period and followed up for a period of at least 05 years i.e. up to 31 November, 2013 and the status of disease of the patients at last contact was recorded. The status of the patients, who had not come for their follow-up, was recorded over telephone.

All BC patients were diagnosed by clinical examination, imaging and fine needle aspiration cytology (FNAC). All patients were underwent surgery (with negative margins), received neoadjuvant and/or adjuvant chemotherapy, radiotherapy and hormone therapy as appropriate. OS was defined as the time from the date of diagnosis to either the date of last follow-up or death. DFS was defined as the time from the date of diagnosis till the date when presence of disease was first recorded.

Most of the patients 237(45.8%) received FAC intravenous chemotherapy. FAC regime consisted of Inj. 5 Fluro-uracil 600mg/m^2, Inj. Adriamycin (Doxorubicin) 60mg/m^2, and Inj. Cyclophosphamide 600mg/m^2 per cycle. Total 6 cycles of chemotherapy was given with a gap of 3 weeks between 2 cycles. 138(26.6%) patients received TAC regime chemotherapy regime. TAC regime consisted of Inj. Docetaxel 75mg/m^2, Inj. Doxorubicin 50mg/m^2 and Inj. Cyclophosphamide 500mg/m^2 per cycle. Due to low economical background 143(27.6%) patients received CMF chemotherapy regime. CMF regime consisted of Inj. 5 Fluro-uracil 600mg/m^2, Inj. Methotrexate 50mg/m^2 and Inj. Cyclophosphamide 600mg/m^2 per cycle. Total 6 cycles of chemotherapy was given with a gap of 4 weeks between 2 cycles. The hormone therapy commonly used along with chemotherapy was Tamoxifen 20mg/day for 5 years if the patient was pre-menopausal, ER and/or PR positive and HER2 negative; if the patient was post-menopausal then Letrozole 2.5 mg/daily was given for 5 years, instead of Tamoxifen 465(89.8%) and 53(10.2%) of the patients were received radiotherapy through Telecobalt and Dual Energy Linear Accelerator respectively.

After completion of treatment, follow-up was conducted at 2-month interval for 1st year and at 3-month intervals from 1 to 5 years, and at 6-month intervals thereafter. During follow up, at every visit, clinical examination was done and detailed history was taken. Routine blood and liver function tests were done in every 6 months. X-ray chest and USG of the whole abdomen was done annually. If symptoms suggestive of cerebral/ skeletal metastases were present, then CT scan of brain/ whole body bone scan was done.

Histology and immunohistochemistry

At the time of investigation BC tumors were fixed in 10% neutral-buffered formalin for 24 hours, and the measurement of tumor size was performed. Then the lymph nodal status, LVI, PNI and grade was determined by sectioning the tumor embedded with paraffin. The histological grades were measured by the modified Bloom-Richardson Grading System. The immunohistochemistry was performed by standard methods.

Statistical analysis

Statistical Analysis was performed with help of Epi Info (TM) 3.5.3. EPI INFO is a trademark of the Centers for Disease Control and Prevention (CDC). Z-test (Standard Normal Deviate) was used to test the significant difference between two proportions. Cox-proportional hazard ratio is used. The Kaplan-Meier survival method was used to estimate the survival and Log-rank test was used to compare the survival curves with respect to different parameters under study. $p<0.05$ was considered statistically significant.

Results

The information regarding age distribution, religion, marital status, menopausal status, family history of BC, co-morbid conditions, laterality, affected quadrant of breast, clinical stage are shown (Table 1). The information regarding pathological and immunohistochemical parameters are shown (Table 2). The information regarding parameters related to treatment is shown (Table 3). The information regarding Comparing LVI and PNI status with clinicopathological parameters is shown (Table 4)

The mean age (mean±standard error (s.e.) of the BC patients at presentation was 46.55±0.51 years with range 22-85 years and the median age was 45.0 years. Most of the patients 163 (31.5%) were in the age group 40-49 years and also patients in the age group 30-59 years 413(79.72%) were significantly higher than other age groups ($Z=8.41$;$p<0.01$). Proportion of Hindu 378(73.0%) were significantly higher than other religion ($Z=6.51$; $p<0.01$). Proportion of married 516(99.6%) were significantly higher than unmarried ($Z=14.02$; $p<0.01$). There was no significant difference between pre-menopausal 247(47.7%) and post-menopausal patients 271(52.3%) ($Z=0.65$; $p>0.05$). Only 5(0.9%) of the patients had family history of BC. 29(5.6%) were having diabetes, 54(10.4%) were hypertensive, 22(4.2%) were having diabetes as well as hypertension and 413(79.8%)

Table 1. Distribution of Different Parameters

Parameters	Number of patients (%)	p-value
Age groups (in years)		
<30	24 (4.6)	<0.01
30-39	130 (25.1)	
40-49	163 (31.5)	
50-59	120 (23.2)	
60-69	61 (11.8)	
>69	20 (3.9)	
Religion		
Hindu	378 (73.0)	<0.01
Muslim	132 (25.6)	
Christian	5 (0.9)	
Others	3 (0.5)	
Marital Status		
Unmarried	2 (0.4)	<0.01
Married	516 (99.6)	
Menopausal Status		
Pre-menopausal	247 (47.7)	>0.05
Post-menopausal	271 (52.3)	
Family History of BC		
Yes	5 (1.0)	<0.01
No	513 (99.0)	
Co-morbid conditions		
Diabetes	29 (5.6)	>0.05
Hypertensive	54 (10.4)	
Diabetes and Hypertensive	22 (4.2)	
No	413 (79.8)	
Laterality of tumor		
Right	239 (46.1)	<0.05
Left	274 (52.9)	
Bilateral	5 (1.0)	
Affected quadrant of breast		
Upper Outer	307 (59.3)	<0.01
Upper Inner	64 (12.4)	
Lower Outer	60 (11.6)	
Lower Inner	81 (15.6)	
Central	6 (1.2)	
Clinical stage		
0	2 (0.4)	<0.01
I	23 (4.4)	
II	200 (38.6)	
III	193 (37.3)	
IV	100 (19.3)	

were not having any co-morbid condition. There was no significant difference between the laterality of the tumor (Z=0.94; p>0.05). Only 5(0.9%) of the patients had bilateral tumors. Proportion of affected upper outer quadrant of breast 307(59.3%) were significantly higher than others (Z=2.63; p<0.01). Only 25(4.8%) of the patients were in early stage of their diseases at the time of first visit which was significantly lower than other stage (Z=12.78; p<0.01). 293(56.6%) of the patients were in stage-III and stage-IV.

Only 49(9.5%) of the patients were having tumor size <2cm which was significantly lower than other size (Z=11.45; p<0.01). 134(25.9%) of the patients were having tumor size ≥5.0cm. Only 15(2.9%) were node negative patients which was significantly lower than other size (Z=13.32; p<0.01). 80(15.4%) of the patients were more than 9 positive lymph node metastasis. Most of the patients 503(97.1%) were having invasive ductal carcinoma which was significantly higher than other type of histopathology (Z=13.32; p<0.01). Most of the patients 292(56.4%) were having grade-3 tumors which was significantly higher than grades (Z=1.98;p<0.05). 155(29.9%) and 22(4.2%) were having positive LVI and PNI respectively. 289(55.8%) and 288(55.6%) of the patients were having negative estrogen receptor (ER) and progesterone receptor (PR) status respectively. 66(12.7%) of the patients were having human epidermal growth factor receptor (HER-2/neu) positive status.

Table 2. Pathological and Immunohistochemical Parameters

Parameters	Number of patients (%)	p-value
Tumor Size (in cm)		
<1.9	49 (9.5)	<0.01
2.0-4.9	335 (64.7)	
≥5.0	134 (25.9)	
Node		
Negative	15 (2.9)	<0.01
1-3	68 (13.1)	
4-9	355 (68.5)	
≥10	80 (15.4)	
Histopathology		
Ductal Carcinoma insitu	1 (0.2)	<0.01
Invasive Ductal Carcinoma	503 (97.1)	
Lobular Carcinoma	5 (1.0)	
Medullary Carcinoma	3 (0.6)	
Mucinous Carcinoma	6 (1.2)	
Grade		
1	23 (4.4)	<0.01
2	203 (39.2)	
3	292 (56.4)	
Lymphovascular invasion		
Positive	155 (29.9)	<0.01
Negative	363 (70.1)	
Perineural invasion		
Positive	22 (4.2)	<0.01
Negative	496 (95.8)	
Estrogen Receptor		
Positive	229 (44.2)	<0.01
Negative	289 (55.8)	
Progesterone Receptor		
Positive	230 (44.4)	<0.01
Negative	288 (55.6)	
Human epidermal growth factor receptor		
Positive	66 (12.7)	<0.01
Negative	452 (87.3)	

Table 3. Parameters Related to Treatment

Parameters	Number of patients (%)	p-value
Type of Surgery		
Breast Conservation Surgery	22 (4.2)	<0.01
Modified Radical Mastectomy	490 (94.6)	
Wide Local Excision	6 (1.2)	
Chemotherapy		
CMF	143 (27.6)	<0.01
FAC	237 (45.8)	
TAC	138 (26.6)	
Radiotherapy		
Telecobalt	465 (89.8)	<0.01
Dual Energy Linear Accelerator	53 (10.2)	
Hormonethearapy		
Positive	229 (44.2)	<0.01
Negative	289 (55.8)	

Table 4. Comparing positive LVI and PNI status according to Clinicopathological Parameters

Clinicopathological Parameters		LVI		p-value	PNI		p-value
		Negative n(%)	Positive n(%)		Negative n(%)	Positive n(%)	
Menopausal Status	Pre	179(72.5)	68(27.5)	0.256	236(95.5)	11(4.5)	0.824
	Post	184(67.9)	87(32.1)		260(95.9)	11(4.1)	
Stage	0	2(100.0)	0(0.0)	0.116	2(100.0)	0(0.0)	0.959
	I	18(78.3)	5(21.7)		22(95.7)	1(4.3)	
	II	137(68.5)	63(31.5)		192(96.0)	8(4.0)	
	III	40(58.8)	28(41.2)		66(97.1)	2(2.9)	
	IV	166(73.8)	59(26.2)		214(95.1)	11(4.9)	
Tumor Size (cm)	≤2.0	90(84.9)	16(15.1)	<0.001	102(96.2)	4(3.8)	0.049
	>2.0-≤5.0	219(67.8)	104(32.2)		313(96.9)	10(3.1)	
	>5.0	54(60.7)	35(39.3)		81(91.0)	8(9.0)	
Grade	I	15(65.2)	8(34.8)	0.787	19(82.6)	4(17.4)	0.003
	II	145(71.4)	58(28.6)		198(97.5)	5(2.5)	
	III	203(69.5)	89(30.5)		279(95.5)	13(4.5)	
Lymph Node Metastasis	0	14(93.3)	1(6.7)	<0.001	15(100.0)	0(0.0)	0.15
	1-3	58(85.3)	10(14.7)		65(95.6)	3(4.4)	
	4-9	244(68.7)	111(31.3)		343(96.6)	12(3.4)	
	≥10	47(58.8)	33(41.3)		73(91.3)	7(8.8)	
ER	Negative	172(70.5)	72(29.5)	0.846	234(95.9)	10(4.1)	0.874
	Positive	191(69.7)	83(30.3)		262(95.6)	12(4.4)	
PR	Negative	172(70.8)	71(29.2)	0.742	234(96.3)	9(3.7)	0.564
	Positive	191(69.5)	84(30.5)		262(95.3)	13(4.7)	
HER-2/neu	Negative	321(71.0)	131(29.0)	0.221	433(95.8)	19(4.2)	0.898
	Positive	42(63.6)	24(36.4)		63(95.5)	3(4.3)	

Most of the patients 490(94.6%) were underwent modified radical mastectomy (MRM), 237(45.8%) were treated with FAC and 465(89.8%) were treated with radiotherapy through telecobalt. 229(44.2%) were administered hormone therapy.

LVI positive tumors were seen in a higher proportion of post-menopausal patients [87(32.1%)] than in pre-menopausal patients [68 (27.5%)] (p=0.256). According to the stage of disease, 5(21.7%) patients in stage I, 63(31.5%) patients in stage II, 28(41.2%) patients in stage III and 59(26.2%) patients in stage IV had LVI positive tumors (p=0.116). 35(39.3%) patients with large (>5cm) tumors, 104(32.2 %) with size (between >2cm to ≤5cm) tumors and 5(21.7%) with small (≤2cm) tumors were LVI positive and this relation was observed to be statistically significant (p<0.001). In grade I 8(34.8%) out of 23 patients, in grade II 58(28.6%) out of 203 patients, in grade III 89(30.5%) out of 292 patients had LVI positive tumors (p=0.787). Patients with lymph node negative tumors 1(6.7%), 1-3 lymph nodes 10(14.7%), 4-9 lymph nodes 111(31.3%) and >9 lymph nodes metastasis 33(41.3%) were found with LVI positive tumors and this was statistically significant (p<0.001). LVI positive was seen 83(30.3%) in ER positive tumors and 72(29.5%) ER negative tumors (p=0.846). LVI positive was seen 84(30.5%) in PR positive tumors and 71(29.2%) PR negative tumors (p=0.742). LVI positive was seen 24(36.4%) in HER-2/neu positive tumors and 131(29.0%) HER-2/neu negative tumors (p=0.221). PNI positive tumors were 11(4.1%) for post-menopausal patients and 11(4.5%) for pre-menopausal patients (p=0.824). According to the stage of disease, 1(4.3%) patients in stage I, 8(4.0%) patients in stage II, 2(2.9%) patients in stage III and 11(4.9%) patients in stage IV had PNI positive tumors (p=0.959). 8(9.0%) patients with large (>5cm) tumors, 10(3.1 %) with size (between >2cm to ≤5cm) tumors and 4(3.8%) with small (≤2cm) tumors were PNI positive and this association was statistically significant (p=0.049). The histological grades were measured by the modified Bloom-Richardson Grading System. In grade I 4(17.4%) patients, in grade II 5(2.5%) patients, in grade III 13(4.5%) patients had PNI positive tumors and this was statistically significant (p=0.003). Patients with 1-3 lymph nodes 3(4.4%), 4-9 lymph nodes 12(3.4%) and >9 lymph nodes metastasis 7(8.8%) were found with PNI positive tumors (p=0.150). PNI positive tumors were 12(4.4%) in ER positive patients (p=0.874). PNI positive tumors were 13(4.7%) in PR positive patients (p=0.564). PNI positive tumors were 3(4.3%) in HER-2/neu positive patients (p=0.898).

Cox-proportional hazard ratio showed that women with a LVI positive were at increased risk of death from BC [adjusted hazard ratio=8.50, 95% confidence interval (CI: 5.68, 12.73); p<0.001]. But no significant risk was found for PNI positive cases [adjusted hazard ratio=1.12, 95% confidence interval (CI: 0.63, 1.98); p=0.684].

The mean (±s.e.) and median overall survival time of all the patients was 51.38±0.70 months and 60 months respectively. The mean (±s.e.) and median disease free survival time of all the patients was 48.27±0.69 months and 54 months respectively.

The mean (±s.e.) and median overall survival of the LVI positive patients were 39.42±1.51 months and 34 months respectively. However, the mean (±s.e.) and median overall survival of the LVI negative patients were 56.49±0.58 months and 58 months respectively. Poor overall survival pattern was observed for LVI positive patients and Log-rank test showed that there

was significant difference in overall survival between LVI positive and LVI negative patients (Log-rank Statistic-168.32; p<0.01) (Figure 1).

The mean (±s.e.) and median overall survival of the PNI positive patients were 37.59±4.21 months and 34 months respectively. However, the mean (±s.e.) and median overall survival of the PNI negative patients were 52.00±0.69 months and 57 months respectively. Poor overall survival pattern was observed for PNI positive patients and Log-rank test showed that there was significant difference in overall survival between PNI positive and PNI negative patients (Log-rank Statistic-24.03; p<0.01) (Figure 2).

The mean (±s.e.) and median disease free survival of the LVI positive patients were 33.71±0.66 months and 32 months respectively. However, the mean (±s.e.) and median disease free survival of the LVI negative patients were 54.49±0.66 months and 56 months respectively. Poor disease free survival pattern was observed for LVI positive patients and Log-rank test showed that there was significant difference in disease free survival between LVI positive and LVI negative patients (Log-rank Statistic-205.43; p<0.01) (Figure 3).

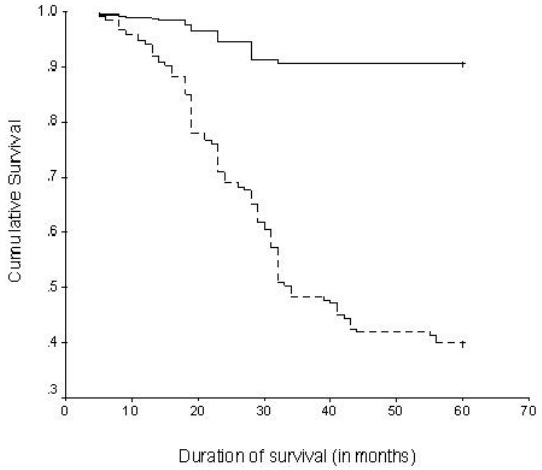

Figure 1. Kaplan-Meier Survival Curve Showing Overall Survival for LVI Positive and LVI Negative Patients

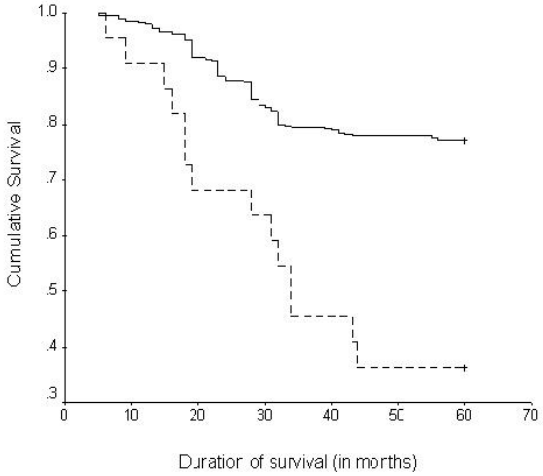

Figure 2. Kaplan-Meier Survival Curve Showing Overall Survival for PNI Positive and PNI Negative Patients

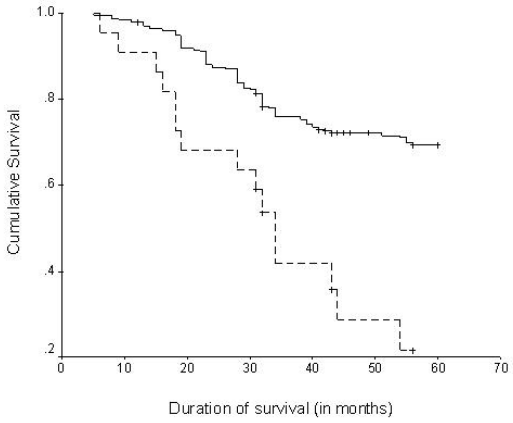

Figure 3. Kaplan-Meier Survival Curve Showing Disease Free Survival for LVI Positive and LVI Negative Patients

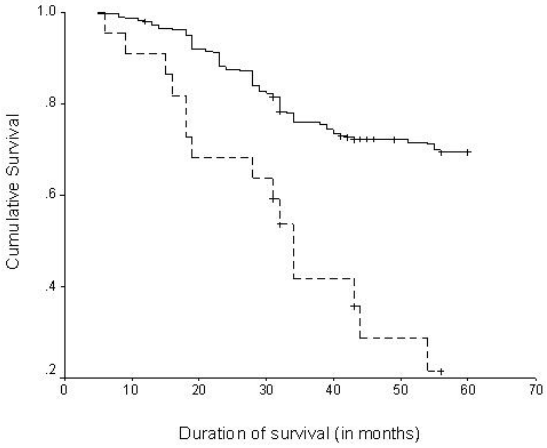

Figure 4. Kaplan-Meier Survival Curve Showing Disease Free Survival for PNI Positive and PNI Negative Patients

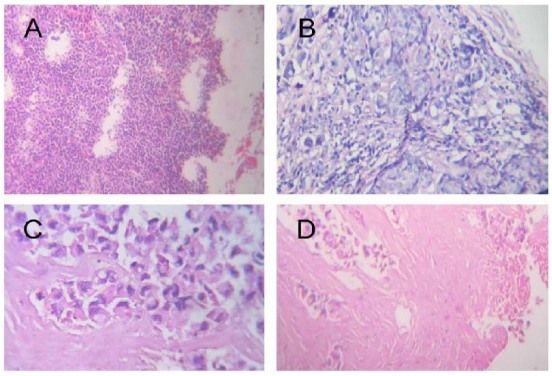

Figure 5. A) No Lymph Node Metastasis, B) Lymph Node Metastasis, C) No Lymphovascular Invasion, D) Lymphovascular Invasion

The mean (±s.e.) and median disease free survival of the PNI positive patients were 32.09±3.26 months and 31 months respectively. However, the mean (±s.e.) and median disease free survival of the PNI negative patients were 48.99±0.72 months and 55 months respectively. Poor disease free survival pattern was observed for PNI positive patients and Log-rank test showed that there was significant difference in disease free survival between PNI positive and PNI negative patients (Log-rank Statistic-

22.95;p<0.01) (Figure 4).

Discussion

Higher percentage of upper outer quadrant tumor of breast is observed in western population (Lee, 2005). We found that carcinoma of upper outer quadrant is greater amount (59.3%) compare with other quadrant in breast tissue. Various studies have suggested that 5-year survival in BC is influenced by tumor size, histological grade, stage of the disease and lymph node involvement (Carter et al., 1989; Henson et al., 1991). Advance stage of breast carcinoma was presented more frequent in India compare with early stage (Sarkar et al., 2013). We observed that most of the patients were in advanced stage of their diseases at first visit and this is happening in many developing countries including India. In India, oncologists who are trained in breast surgery are handful in number. The general medical practitioners need to be trained and oriented in detecting BC. 293 (56.6%) of the patients were in stage-III and stage-IV. Thus the present case series had a poor survival rate, primarily due to the advancement of the disease prior to any cancer directive treatment. Tumor size of BC patients was the predictor for survival (Michaelson et al., 2002). We found that 469 (90.5%) patients were in large size tumor (>1.9cm). 503(97.1%) patients in our study were having invasive ductal carcinoma, a pattern consistent with other studies. The aim of breast surgery in semi-advanced BC should be to eradicate the tumors so that the adjuvant therapy will be optimally effective.

Prognosis of BC depends on nodal status, tumor size and stage of the disease (Michaelson et al., 2002). Various studies have been focused on the identification of characteristics of primary tumors predictive of lymph node involvement (Ferno, 1998; Thames et al., 1999). The invasion and metastasis of tumor cells are major cause of poor prognosis and death of the patient (Schnitt, 2001; Sebastian et al., 2004; Irianiwati et al., 2013). Axillary lymph node metastasis involvement at the time of surgery was most important predictor of prognosis in BC. Approximately 97.1% of BC patients had positive lymph node metastasis and 155(29.9%) of BC patients are positive LVI. We observed that lymphangiogenesis, LVI and PNI were the poor survival factor in BC. Christine Desmedt et al. suggested that the gene expression modules associated with key biological processes in BC tumorigenesis such as proliferation, tumor invasion, immune response, angiogenesis and apoptosis act via estrogen and HER2 signaling (Desmedt et al., 2008). The invasion and metastasis of tumor cells are important biologic features of BC patients which is one of the main causes for poor prognosis and death from BC. Also axillary lymph node involvement at time of surgery is the most significant and durable prognostic factor in BC patients. Like western data we observed that poor OS and DFS was found in LVI positive tumors compare to LVI negative tumors. In this study, we investigated the impact of LVI and PNI on the prognosis in BC patients. Poor overall survival as well disease free survival pattern was observed for LVI positive patients as compared with LVI negative patients who were found statistically significant through Log-rank test. Also poor OS as well DFS pattern was observed for PNI positive patients as compared with PNI negative patients which was found statistically significant as per Log-rank test.

This study looks into the distribution pattern of survival of BC patients in eastern India with their respective status of LVI and PNI. Late detection of BC led to LVI as well as PNI positivity which have been established in this study as a leading cause of poor prognosis of BC patients. Thus we advocate incorporating the status of LVI and PNI into breast cancer staging system. Also as per the results of this study a large number of BC patients in this region were diagnosed at advanced stage of their diseases. It reveals that lack of awareness regarding the symptoms of BC among the women is still prevailing in this region.

Acknowledgements

It is our great pleasure and proud privilege to express our gratitude to Dr. Arnab Gupta, Director and Dr. Apurba Ghose, Department of Surgical Oncology, Saroj Gupta Cancer Centre and Research Institute, Kolkata-700063, West Bengal. They constantly helped us in completing this work. Special thanks to our patients and relatives of the patients for their continuing co-operation.

References

Carter CL, Allen C, Henson DE (1989). Relation of tumour size, lymph node status, and survival in 24,740 BC Cases. *Cancer*, **63**, 181-7.

Cooke T, Reeves J, Lanigan A, Stanton P (2001). HER2 as a prognostic and predictive marker for breast cancer. *Ann Oncol*, **1**, 23-8.

Das S, Sen S, Mukherjee A, Chakraborty D, Mondal PK (2012). Risk factors of breast cancer among women in eastern India: a tertiary hospital based case control study. *Asian Pac J Cancer Prev*, **13**, 4979-81.

Desmedt C, Haibe-Kains B, Wirapati P, et al (2008). Biological process associated with breast cancer clinical outcome depend on the molecular subtypes. *Clin Cancer Res*, **14**, 5158-65.

Donegan WL (1997). Tumor-related prognostic factors for breast cancer. *CA Cancer J Clin*, **47**, 28-51.

Ferlay J, Shin HR, Bray F, et al (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*, **127**, 2893-917.

Ferno M (1998). Prognostic factors in breast cancer: a brief review. *Anticancer Res*, **18**, 2167-71.

Henson DE, Ries L, Freedman LS, Carriaga M (1991). Relationship among outcome, stage of disease, and histologic grade for 22,616 cases of breast cancer. The basis for a prognostic index. *Cancer*, **68,** 2142-9.

Jana D, Das S, Sarkar DK, et al (2012). Role of nuclear factor-ϰb in female breast cancer: a study in Indian patients. *Asian Pac J Cancer Prev*, **13**, 5511-5.

Jana D, Mandal S, Mukhopadhyay M, et al (2012). Prognostic significance of HER-2/neu and survival of breast cancer patients attending a specialized breast clinic in Kolkata, Eastern India. *Asian Pac J Cancer Prev*, **13**, 3851-5.

Karak SG, Quatrano N, Buckley J, Ricci A Jr (2010). Prevalence and significance of perineural invasion in invasive breast carcinoma. *Conn Med*, **74**, 17-21.

Khokhar A (2012). Breast cancer in India: Where do we stand

and where do we go? *Asian Pac J Cancer Prev*, **13**, 4861-6.
KK Ma, Wai Wang Chau, Connie HN Wong, et al (2012). Triple negative status is a poor prognostic indicator in chinese women with breast cancer: a ten year review. *Asian Pac J Cancer Prev*, **13**, 2109-14.
Laguens G, Coronato S, Di Girolamo (2006). Biomarkers in breast cancer. *CEJ Med*, **1**, 330-47.
Lee AH (2005). Why is carcinoma of the breast more frequent in the upper outer quadrant? a case series based on needle core biopsy diagnoses. *Breast*, **14**, 151-2.
Michaelson JS, Silverstein PM, Wyatt J, et al (2002). Predicting the survival of patients with breast carcinoma using tumor size. *Cancer*, **95**, 713-23.
Muhammadnejad S, Muhammadnejad A, Haddadi M, et al (2013). Correlation of microvessel density with nuclear pleomorphism, mitotic count and vascular invasion in breast and prostate cancers at preclinical and clinical levels. *Asian Pac J Cancer Prev*, **14**, 63-8.
Nathanson SD, Zarbo RJ, Wachna DL, et al (2000). Microvessels that predict axillary lymph node metastases in patients with breast cancer. *Arch Surg*, **135**, 586-93.
Noel W, Joseph PS, Willian RW, Judah F (1991). Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. *N Engl J Med*, **324**, 1-8.
Sarkar DK, Jana D, P. S. Patil, et al (2013). Role of NF-ϰB as a prognostic marker in breast cancer: a pilot study in Indian patients. *Indian J Surg Oncol*, **13**, 0234-8.
Schnitt SJ (2001). Traditional and newer pathologic factors. *J Natl Cancer Inst*, **30**, 22-6.
Sebastian FS, Guenther B, Aumayr K , et al (2004). Prognostic value of lymphangiogenesis and lymphovascular invasion in invasive breast cancer. *Ann Surg*, **240**, 306-12.
Sen U, Sankaranarayanan R, Mandal S, et al (2002). Cancer patterns in eastern India: The first report of the Kolkata cancer registry. *Int J Cancer*, **100**, 86-91.
Tan LGL, Tan YY, Heng D, Chan MY (2005). Predictors of axillary lymph node metastases in women with early breast cancer in Singapore. *Singapore Med J*, **46**, 693-7.
Thames HD, Buchholz TA, Smith CD (1999). Frequency of first metastatic events in breast cancer: implications for sequencing of systemic and local-regional treatment. *J Clin Oncol*, **17**, 2649-58.
Widodo I, Ferronika P, Harijadi A, et al (2013). Clinicopathological signifcance of lymphangiogenesis and tumor lymphovascular invasion in Indonesian breast cancers. *Asian Pac J Cancer Prev*, **14**, 997-1001.