IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 18, Issue 1 Ser. 10 (January. 2019), PP 10-15
www.iosrjournals.org

A Study on Importance of Molecular Subtyping As a Prognostic Indicator of Breast Carcinoma

Dr Anirban Das[1], Dr Chiranjit Mukherjee[2], Dr Arnab Chakraborty[3], Dr Amit Kumar Das[4]

[1](Tutor, Department of Surgery, NRSMC, Kolkata, West Bengal,India)
[2](Specialist Medical Officer, Surgery, Bishnupur Multi/Super Speciality Hospital,Bankura, West Bengal,India)
[3](Specialist Medical Officer, Surgery, Deben Mahato Sadar Hospital, Purulia, West Bengal, India)
[4](Tutor, Department of Surgery, BSMC, Bankura, West Bengal, India)
Corresponding Author: Dr Amit Kumar Das (Tutor, Department of Surgery, BSMC, Bankura, West Bengal, India)

Abstract
BACKGROUND*: Breast cancer (BC) is no longer seen as single disease but rather multifaceted disease comprised of distinct biological subtypes with diverse natural history. Molecular subtyping of Breast Cancer helps in prediction of disease outcome and therapy.*
METHODS*: 82 patients with IDC type BC, from January ,2014 to August, 2018 in IPGME&R, Kolkata were taken in this study group. Receptors status analyzed by Immuno-histochemistry study and correlated with clinico-pathological parameters, OS and DFS studied on follow up. Exposure to different risk factors also assessed.*
RESULT: *Most of the patients in this study were between 51-60 yrs of age. Triple negative subtype was most prevalent as compared to other subtypes. Disease free survival (DFS) was best in Lumina B (80%) followed by Luminal A (77.85). Her2 neu group had highest recurrence rate (23%), comparable with Triple negative groups (15.2%). Overall survival was best among Luminal A (94.4%).*
CONCLUSION*: . Luminal B and LuminalA subtypes showed good DFS and OS as compared to Her2 neu and Triple negative subtypes.*
KEY WORDS: *Breast Cancer(BC), Overall Survival(OS), Disease Free Survival (DFS), Molecular Subtypes.*

Date of Submission: 05-01-2019 Date of acceptance: 21-01-2019

I. Introduction

Breast cancer(BC) is no longer seen as single disease but rather multifaceted disease comprised of distinct biological subtypes with diverse natural history[1] . There are various prognostic factors that are used to predict the outcome and to follow treatment plan, eg. Tumour size, tumour grade, Ki67 value, NPI, axillary lymph node status [2][3]. There are also numerous molecular markers that are found to affect breast cancer outcomes, including steroid hormone receptor pathway (ER and PR), Human Epidermal Receptor pathway (HER family), Cycline dependent kinase (CDKs), Cycloxigenase-2(COX-2), Transforming Growth Factor-g(TGF-g)etc.Molecular subtypes are done by analyzing the various receptor status such as Hormonal like Estrogens (ER), Progesterone (PR), Epidermal growth factor like Her -2/neu as expressed by the tumour cells in Immunohistochemistry (IHC) or Genetic array testing [3]. After analyzing these receptors status, they are categorized into four subtypes as follows:-

Molecular subtypes	Receptor status found
Luminal A	ER +/PR +/Her2 - ER -/PR +/Her2 - ER +/PR -/Her2 -
Luminal B	ER +/PR +/Her2 + ER -/PR +/Her2 + ER +/PR -/Her2 +
Her-2/neu type	ER -/PR -/Her2 +
Triple negative/ Basal type	ER -/PR -/Her2 -

II. Aims and objectives

Aims of our study were

1. To analyze the demographic and risk variables in study population of BC.
2. To observe the prevalence of various molecular subtypes of BC.

3. To study the relation between molecular subtypes and the established prognostic factors such as tumor size, tumor grade , margin status, LN involvement.
4. To study the NPI, overall survival (OS) and disease free survival (DFS) in different molecular subtypes.

III. Materials and methods

The study was carried out in the Department of Surgery and the Comprehensive Breast Service Clinic at IPGME&R/ S.S.K.M. Hospital, Kolkata, West Bengal, India.

Study Design- Prospective, Observational.

Study Location: Department of Surgery, and the Comprehensive Breast Service Clinic at IPGME&R/ S.S.K.M. Hospital, Kolkata. This is a Tertiary Teaching Institute of West Bengal, India.

Study Duration: January, 2014 to August, 2015 with at least 1 year 6 months follow up after therapy.

Sample Size- 82 Patients.

Sample Selection and Methods of Study: All the post-operative patients attending the above departments-

Inclusion criteria : All the female patients with a diagnosis of Infiltrating Ductal carcinoma(IDC) of Breast, previously untreated by CT,RT,HT or combination of any.

Exclusion criteria : All the patients with diagnosis of other benign breast diseases, Male breast cancer, breast cancer previously treated with CT,RT,HT or any combination therapy, other pathological types of BC like LC, MC, etc., Patients who did not receive complete treatment or lost to follow-ups were excluded.

Study technique: Clinical evaluation of the patients as per the inclusion criteria and evaluation of the clinico-radiological, histopathological and Immunohistochemistry features were done. Correlation of ER, PR, HER-2/neu status was done with the other parameters. Information like date and location of recurrence, date of death and length of survival were studied.

Chemotherapy treatment and Follow up protocol:

All patients were admitted in the hospital and received HT/ CT under direct supervision. The most common regime used for intravenous chemotherapy was the FAC(5Fluorouracil, Inj. Adriamycin ,Inj. Cyclophosphamide) regimen. Total 6 cycles of chemotherapy were given with a gap of 3 weeks between 2 cycles. The HT was given with Tamoxifen 20 mg/ day for 5 years. In post menopausal patients, Tab Letrozole 2.5 mg/ Day was given for 5 years instead of Tamoxifen. The Luminal A and Luminal B subtypes were treated with both FAC regimen and HT. Some of Her2 positive patients, who could afford, received Trastuzumab (T) 4 mg/ Kg body weight (FAC-HT-T).Some triple negative patients received TAC(Inj. Paclitaxel, Inj. Doxorubicin, Inj. Cyclophosphamide) regimen but due to economical constraints, most of the patients received chemotherapy with FAC. Majority of the patients belonging to the nonluminal Her 2 positive only group received FAC/TAC.

Each patient was followed up at 2-monthly interval for 1st year and at 3-monthly intervals from next year. The time period from the date of surgery to the date of death from any cause was considered as the overall survival (OS) and to the date of recurrence of disease was considered as the disease free survival (DFS). On follow-up, clinical examination, routine blood and liver function test (LFT) examination were done every 6 months. X ray chest and USG of the abdomen and pelvis was done annually. If symptoms suggestive of any cerebral, skeletal metastasis were present, then CT scan brain, CT of abdomen with Pelvis, whole body bone scan were done within the 1 and ½ years follow-up period.

Statistical Analysis:

For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by Epi Info (TM) 3.5.3. EPI INFO is a trademark of the Centers for Disease Control and Prevention (CDC).

The Kaplan-Meier survival analyses were carried out for overall survival and disease free survival. The Kaplan-Meier method followed by log-rank test was used to compare the survival patterns of different molecular subtypes.

IV. Result and analysis

Most of the patients were in the age group 51- 60 years (45.1%). Luminal A patients mostly (38.9%) presented in age group 41-50 years and triple negative mostly presented in 51-60 years age group (58.7%). Her 2/neu presented in 41-50 years age group (61.5%)(p=0.0166).

Table-1: Showing association between age of patients and molecular subtypes

Age (Years)	Luminal A	Luminal B	Triple Negative	HER2 +	TOTAL
≤40	1	1	0	1	3
Row %	33.3	33.3	0.0	33.3	100.0
Col %	5.6	20.0	0.0	7.7	3.7

41-50	7	1	10	8	26
Row %	26.9	3.8	38.5	30.8	100.0
Col %	38.9	20.0	21.7	61.5	31.7
51-60	5	1	27	4	37
Row %	13.5	2.7	73.0	10.8	100.0
Col %	27.8	20.0	58.7	30.8	45.1
61-70	5	1	7	0	13
Row %	38.5	7.7	53.8	0.0	100.0
Col %	27.8	20.0	15.2	0.0	15.9
71-80	0	1	2	0	3
Row %	0.0	33.3	66.7	0.0	100.0
Col %	0.0	20.0	4.3	0.0	3.7
TOTAL	18	5	46	13	82
Row %	22.0	6.1	56.1	15.9	100.0
Col %	100.0	100.0	100.0	100.0	100.0

Premenopausal patients mostly presented with Luminal A and Her 2/neu subtype (34.8% each). Postmenopausal patients mostly presented with Triple negative subtypes (69.5%)(p=0.0008).

Table -2: Association between menopausal status of patients and molecular subtypes.

Menopausal status	Luminal A	Luminal B	Triple Negative	HER2 +	TOTAL
Postmenopausal	10	3	41	5	59
Row %	16.9	5.1	69.5	8.5	100.0
Col %	55.6	60.0	89.1	38.5	72.0
Premenopausal	8	2	5	8	23
Row %	34.8	8.7	21.7	34.8	100.0
Col %	44.4	40.0	10.9	61.5	28.0
TOTAL	18	5	46	13	82
Row %	22.0	6.1	56.1	15.9	100.0
Col %	100.0	100.0	100.0	100.0	100.0

Only 2.4% of the study population had evidence of positive family history of BC. 4.9% patients in the study group used OCP for at least 5 years in their life time. Most of the patients had history of early menarche before age of 12 years (69.5%) which is a known risk factor of BC. Most of the patients had delivered their first child at the age group 25- 30 years (57.3%). 41.6% patients of BC had given exclusive breast feeding to their child. 3.5% patients did not offer breast feeding as they had problem in milk output after birth. 48.8% patients had one or two children, 47.6% patients had three or four children.

Table-3: Distribution of Different Risk Factors

Parameters		Frequency	Percent
Family History	No	80	97.6
	Yes	2	2.4
OCP Use	No	78	95.1
	Yes	4	4.9
Age at Menarche	<10 years	1	1.2
	10-12 years	56	68.3
	12-15 years	23	28.0
	>15 years	2	2.4
Age at First Child Birth	<18 years	1	1.2
	18-20 years	3	3.7
	20-25 years	29	35.4
	25-30 years	47	57.3
	>30 years	2	2.4
Breast Feeding	No	3	3.5
	Non Exclusive	45	54.9
	Exclusive	34	41.6
Number of Live Birth	Nil	0	0
	1-2	40	48.8
	3-4	39	47.6
	5 and above	3	3.6

Most of the patients of BC showed triple negative in Immunohistochemistry (56.1%) syudy followed by Luminal A (22%). Least was Luminal B (only 6%).

Table-4: Distribution of BC according to Molecular Subtypes

Molecular subtype	Frequency	Percent
Luminal A	18	22%
Luminal B	5	6%
Her 2/ neu	13	15.9%
Triple Negative	46	56.1%
Total	82	100.0%

Triple negative patients mostly presented in stage III (73.9%) and stage IV (19.6%). Luminal A mostly presented in stage III (88.9%). Luminal B presented mostly in stage II (80%) (p<0.0001). Triple negative patients presented with large tumour size >= 5 cm (76.5%). Luminal A and Her 2/neu presented mostly with moderate tumour size (77.8% and 76.9%) (p=0.0171). Triple negative patients showed maximal nodal involvement, >9 nodes (94.4%) as compared to other subtypes (p<0.0001). Most of the Luminal A patients showed grade 3 (66.7%) at time of diagnosis, Luminal B patients showed either Grade 1 or Grade 2 (40% each) at diagnosis. Triple negative patients showed higher grade (Grade 3 ,67.2%) at presentation (p=0.0021). Luminal A had most favorable (41.7%) NPI(≤5.4). Triple Negative had the worst (61.4%) NPI(> 5.4)(p=0.050).

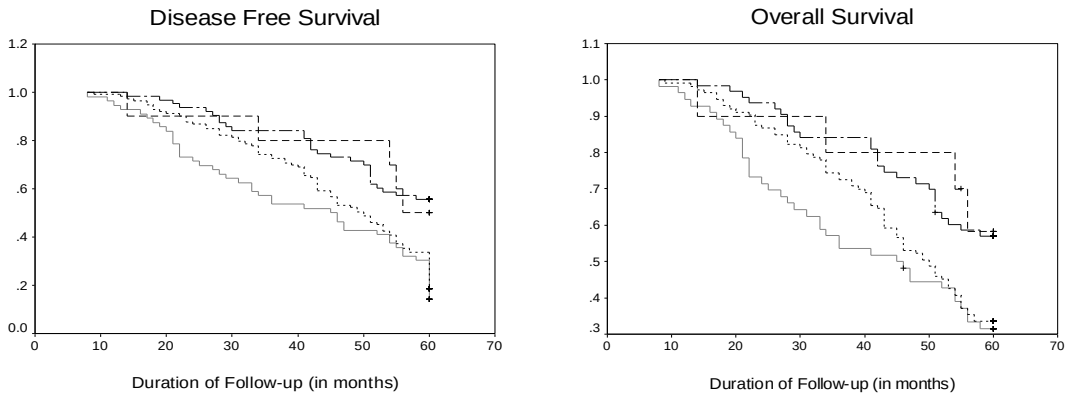
Table-5 : Distribution of Clinico-pathological Parameters with Molecular Subtypes

		Molecular Subtypes			
Clinico-pathological Parameters		Luminal A	Luminal B	Triple Negative	Her2
Stage	I II III IV	1(33.3%) 1(5.9%) 16(30.8%) 0(0.00%)	1(33.3%) 4(23.5%) 0(0.00%) 0(0.00%)	1(33.3%) 2(11.8%) 3465.4%) 9(90.0%)	0(0.00%) 10(58.8%) 2(3.8%) 1(10.0%)
Tumour Size	<2cm 2-4.99cm ≥5.00	1(33.3%) 14(31.1%) 3(8.8%)	1(33.3%) 2(4.4%) 2(5.9%)	1(33.3%) 19(42.2%) 26(76.5%)	0(0.00%) 10(22.2%) 3(8.8%)
Nodal Status	0 1-3 4-9 >9	0(0.00%) 18(62.1%) 0(0.00%) 0(0.00%)	1(50.0%) 4(13.8%) 0(0.00%) 0(0.00%)	1(50.0%) 1(3.4%) 27(81.8%) 17(94.4%)	0(0.00%) 6(20.7%) 6(18.2%) 1(5.6%)
Grade	1 2 3	1(20.0%) 5(26.3%) 12(20.7%)	2(40.0%) 2(10.5%) 1(1.7%)	1(20.0%) 6(31.6%) 39(67.2%)	1(20.0%) 6(10.3%) 6(10.3%)
NPI	≤5.4 >5.4	5(41.7%) 13(18.6%)	2(16.7%) 3(4.3%)	3(25.0%) 43(61.4%)	2(16.7%) 11(15.7%)

Disease free survival (DFS) was best in Lumina B (80%) followed by Luminal A (77.85). Her 2 group had highest recurrence rate (23%) comparable with Triple negative groups (15.2%). Overall survival was best among Luminal A (94.4%) (p=<0.001).

Table-6 : Survival Status according to Molecular Subtypes

Survival status	Luminal A (n=18)	Luminal B (n=5)	Her 2/ neu (n=13)	Triple negative (n=46)	Comparisons
DFS	14 (77.8%)	4 (80%)	3 (23%)	16 (34.8%)	<0.001 (p value)
Relapse	3 (16.6%)	0 (0%)	3 (23%)	7 (15.2%)	
Death	1 (5.6%)	1 (20%)	7 (54%)	23 (50%)	
Total	18 (100%)	5 (100%)	13 (100%)	46 (100%)	



Same percent Luminal A patients were treated with FAC+HT therapy as adjuvant. 80% Luminal B patients were treated with FAC+HT therapy whereas 20% treated with FAC+HT+Trastuzumab therapy. 90% triple negative patients were treated with FAC and 10% were treated with TAC therapy.

Table-7: Status of Adjuvant therapy among patients on follow up

Adjuvant Therapy	Luminal A (n=18)	Luminal B (n=5)	Her 2/ neu (n=13)	Triple Negative (n=46)	Comparisons
FAC	0	0	11 (84.6)	40 (90%)	<0.001 (p value)
TAC	0	0	1 (7.7%)	6 (10%)	
FAC+ HT	18 (100%)	4 (80%)	0	0	
FAC+ HT+ Trastuzumab	0	1 (20%)	0	0	
TAC+ Trastuzumab	0	0	1 (7.7%)	0	
Total	18 (100%)	5 (100%)	13 (100%)	46 (100%)	

V. Discussion

Invasive Duct Carcinoma patients, who had not taken any form of neo-adjuvant therapy were evaluated, so the chance of any change in the molecular sub typing due to pre-existing therapy was negated. Most of the patients were in the age group of 51-60 years . Mean age was 55+- 8 (2SD). Most of the patients were post-menopausal (72%). Only 2.4% of the study population had evidence of positive family history of BC. Only 4.9% patients in the study group used OCP for at least 5 years. Most of the patients had history of early menarche(< 12 years ,69.5%) which was a known risk factor of BC. Most of the patients had delivered their first child at the age group 25-30 years (57.3%). 41.6% patients of BC had followed exclusive breast feeding . All the patients of BC had children in their life. There was no history of any addiction to the study population to either smoking or alcohol.

Most of the patients of BC showed triple negative in Immunohistochemistry (56.1%) study followed by Luminal A (22%). Other study revealed Luminal A was most prevalent[3][4][5][6]. Some study also supported our syudy[7]. Luminal A patients mostly (38.9%) presented in age group 41-50 years and triple negative mostly presented in 51-60 years age group (58.7%). Her 2/neu presented in 41-50 years age group (61.5%).Luminal A mostly presented in stage III(88.9%). Luminal B presented mostly in stage II disease (80%). Triple negative BC presented in advanced stage as compared to other subtypes. Triple negative patients presented with large tumour (76.5%) size and Luminal A , Her 2/neu presented with moderate tumour size (77.8% and 76.9%).Triple negative BC patients showed maximal nodal involvement (94.4%). Most of the Luminal A patients showed grade 3 (66.7%) , Luminal B patients showed either Grade 1 or Grade 2 (40% each) at diagnosis. Luminal A subtype had most favourable NPI 5.4(≤5.4) whereas patients with Triple Negative had the worst (61.4%) NPI (> 5.4). Other study also revealed similar result [8]. DFS was best in Luminal B (80%) followed by Luminal A (77.85). Her2/neu group had highest recurrence rate (23%), comparable with Triple negative groups (15.2%). OS was best among Luminal A (94.4%). Lowest DFS was among Her 2/neu group (23%) and lowest OS was among triple negative group (50%). Lowest DFS in Her 2/neu groups was probably due to lack of use of Trastuzumab due to high cost. Other study showed that triple negative subtype (ER/PR-,Her2-) has the worst overall survival (79%, hazard ratio, 1.8; 95% CI, 1.06-3.2), and worst disease-free survival (73%, hazard ratio, 1.5; 95% CI, 0.8-3.0). Luminal A has better OS (90.3%) and DFS (86.8%)[9][7].

VI. Conclusion

In conclusion, Triple negative subtype was most prevalent and presented with advanced clinico-pathological behavior in contrary to Western studies. Luminal B and Luminal A subtypes showed better DFS and OS as compared to Her2/neu and Triple negative subtypes.

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Dr Amit Kumar Das, "A Study on Importance of Molecular Subtyping As a Prognostic Indicator of Breast Carcinoma." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 18, no. 1, 2019, pp 10-15.