Original article

Hormonal receptor status with relation of subtypes and association of proliferation marker Ki-67 in breast cancer patients; a potential biomarker in patients with breast cancer

Vijay Pratap Singh[1], Rahul Kumar Choudhary[1], Arun Kumar Jha[2], Chandan Kumar Singh[3], Akash Kumar Singh Singh[1], Avinash Pandey[4], Anita Kumari[5], Vishal Mohan Singh[6], Amrita Rakesh[5], Anandita Jha[7], Pranab Kumar Verma[1], Manish Jaipuriyar[1], Shambhwi Sharma[1], Sanjay Kumar[1], Ajeet Kumar Singh[1]

[1] Department of Surgical Oncology, Savera Cancer and Multispeciality Hospital, Patna, India
[2] Department of Clinical Research, Savera Cancer and Multispeciality Hospital, Patna,India
[3] Department of Molecular Cancer Genetics, Savera Cancer and Multispeciality Hospital, Patna, India
[4] Department of Medical Oncology, Savera Cancer and Multipseciality Hospital, Patna, India
[5] Department of Radiation Oncology, Savera Cancer and Multispeciality Hospital, Patna, India
[6] Department of Anaesthesia, Savera Cancer and Multispeciality Hospital, Patna, India
[7] Indira Gandhi National Open University (IGNOU), New Delhi, India

ABSTRACT

Background: Hormone receptor status is an important prognostic and therapeutic tool in breast cancer. The present study aimed to determine the comprehensive analysis of hormone receptor status and Ki-67 in breast cancer patients at a tertiary cancer centre.

Methods: The relationship of subtypes ER+PR+, ER+PR-, HER2 subtype (Luminal HER2; ER+PR+HER2+), and HER2 enriched (ER-PR-HER2+) status with multiple variables were evaluated. The expression of Ki-67 was strongly associated with cancer proliferation and is a known indicator of prognosis and outcome. The study was carried out in Savera Cancer and Multispeciality Hospital, Patna, India.

Result: This study includes a total of (n=979) breast cancer cases diagnosed at our centre, out of which (n=335; 34.22%) cases went through hormonal investigation. Luminal A (31.05%) subtype was the most prevalent, followed by triple negative (20.16%), luminal B (8.4%), and HER2-positive (20.05%). Majority of the tumours were located on right side (n=186; 55.52%), on the left (n=131; 39.1%) and bilateral (n=18; 5.37%) not significant (P <0.0003). As per ECOG performance status criteria 148 (44.17%) patients were on scale 3, 89 (26.56%) – on scale 2 and 24, 37, and 37 – on scale 0, 1 and 4 respectively statistical value (P=0.325). Remarkable finding in this study Ki-67 level was increased in >50% cases of TNBCs which was significant (P=0.05). As per AJCC staging criteria (n=205; 61.19%) cases were in stage III (locally advanced breast cancer). The value was significant (P=0.05). A total of 236 (72.39%) patients are alive with life expectancy of >24 months.

Conclusion: In conclusion, this retrospective study demonstrated a high prevalence rate of triple negative breast cancer at our centre. Majority of cases were of TNBC subtypes. Another remarkable finding of this study was, in majority of triple negative cases Ki-67 level was >50%. Elevated level of Ki-67 indicated the aggressiveness of tumor types.

Key words: breast cancer, hormonal status, triple negative breast cancer, Ki-67

Correspondence:
Dr Vijay Pratap Singh
Department of Surgical Oncology, Savera Cancer and Multispeciality Hospital
Near, R N Singh Road, Rajendra Nagar Over Bridge, Kankarbagh, Patna, Bihar 800020, India
e-mail: vijaypsingh_2000@yahoo.com

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Abbreviations:

AJCC – American Joint Committee on Cancer
BC – Breast Cancer
PARP – Poly-ADP Ribose Polymerase
ECOG – Eastern Cooperative Oncology Group
ER – Estrogen Receptor
HER2 NEU – Human Epidermal Growth Factor Receptor
NED – No Evidence of Disease
HPE – Histopathological Examination
OS – Overall Survival
PR – Progesterone Receptor
ICH–GCP – International Conference on Harmonization – Good Clinical Practices
IHC – Immunohistochemistry
RECIST 1.1– Response Evaluation Criteria in Solid Tumors
TNBC – Triple Negative Breast Cancer
mTNBC – Metastatic Triple Negative Breast Cancer
mTOR – Mammalian Target of Rapamycin

INTRODUCTION

Bihar, a state in eastern India is thickly populated and few other states in India are more populous than most of the countries across the globe. Only 12 nations across the globe have a population more than that of Bihar. Low literacy rate reported from this state about 60–64% only. Approximately 89% of the population are coming from rural areas according to the last census report of the government of India released in 2011 [1]. Breast cancer is considered to be the most common cancer among women and was associated with poor prognosis [2].

Hormonal receptors (ER, PR and Her2 Neu) are well recognised histopathological factors as prognostic and predictive markers and have leaded a revolution in the treatment of BC in this era called immunotherapy [3–5]. An estimated 19.3 million incidence of cancer cases worldwide in 2020 as per the Global Cancer Observatory (GLOBOCAN) [6]. An increase of 2.08 million cancer cases in India, accounting for a rise of 57.5% in 2040 from 2020, predicted by GLOBOCAN [7].

By the year 2030 it is estimated that the burden of BC is expected to rise by 2 million as per the epidemiological studies [8]. The cases are staggering and constantly rising. The latest trends point out that a higher proportion of the disease is occurring at a younger age in Indian women, as compared to the West due to adopting sedentary life styles. Molecular and genetic investigations are very expensive and not available in tier ⅔ cities of India. Clinicians generally tend to rely on the low cost and readily available immunohistochemistry classification of the breast in these countries [9]. India has one of the highest rates of the most aggressive subtype of BC referred to as Triple-Negative Breast Cancer (TNBC) [10]. Multiple factors may account for higher prevalence of TNBC reported by studies conducted among Indian patients with breast cancer. The early age of onset of breast cancer; lifestyle factors, such as diet and obesity; reproductive factors, such as multiparity; socioeconomic status; and screening behaviors may be hypothesized as probable etiology [11, 12]. Another important factor could be a potential genetic susceptibility of Indians to TNBC. According to the National Comprehensive Cancer Network (NCCN) guidelines 2020, the recommended treatment for the mTNBC patients is single drug treatment. Combined chemotherapy is for patients with a high tumor burden, rapid disease deterioration, and visceral crisis of organ metastasis. The most recommended and standard treatment options for mTNBC patients are doxorubicin, liposomal doxorubicin, paclitaxel, capecitabine, gemcitabine, vinorelbine, eribulin, olaparib, talazoparib, cisplatin, carboplatin and atezolizumab combined with nab-paclitaxel. For some circumstances, the recommended treatment options were combination treatments such as doxorubicin/cyclophosphamide (AC), epirubicin/cyclophosphamide (EC), cyclophosphamide/methotexate/fluorouracil (CMF), docetaxel/capecitabine and paclitaxel/bevacizumab [12].

METHODOLOGY

This is a retrospective study of a prospectively maintained database of Hospital Based Cancer Registry Program (HBCR). The breast cancer patients treated at our centre between January 2019 and December 2022. The present study was carried out at Savera Cancer and Multispeciality Hospital, Patna, India.

Inclusion criteria

1. Female patients between the age groups of 18 to 90 years.
2. Patient must have histopathological or cytological confirmed breast cancer.
3. Patients with life expectancy of at least 12 months.
4. Patients willing to take up calls during the time of telephonic follow-ups.
5. Subject is willing and able to comply with the protocol for the duration of the study.

Exclusion criteria

Patients unwilling to follow protocol requirements.

Antibodies and buffers were used for analysis

Rabbit Monoclonal Antibody for ER (PR042-3ML; Vol – 3 ml), PR (PR068-3ml), HER2 Neu (PR047-3ML) and Mouse Monoclonal Antibody were used in Ki67-MIB-1 (PM210-3ML). PloyExcel HRP/DAB

detection system contains of PolyExel H_2O_2, PloyExcel Target Binder, PolyExcel Poly HRP, PolyExcel Stunn DAB-Chromogen, Stunn DAB buffers (PEH002: 6ML, Vol: 6 ml), Tris-EDTA Buffer – 50X Concentrated (PS009-100 ml, Vol: 100 ml) and Immuno Wash Buffer – 25X Concentrated (PS006-500ml; Vol: 500 ml).

This study was conducted in accordance with ICH–GCP guidelines. Ethics committee approval was not required due to non-interventional study. Medical records were referred for culling out the data and extracting patient information. Data was collected and collated related to demographic profile, tumor details, pathologic assessments, treatment, follow-up and survival status information. Status at last follow-up was confirmed either through medical records or telephonically. IHC evaluations were performed under the supervision of expert team of pathologist. For intermediate/ equivocal HER2/neu, cases were referred for the FISH assay in the referral laboratories as the facility for FISH was not available in our institute.

Statistical analysis

All data were recorded and analyzed on Microsoft Excel 2007 and XLSTAT software. The P value level of 0.05 was used to assess statistical significance.

switched to molecular level. Patients were getting the advantages of targeted and immunotherapy.

Ki-67 evaluation

The Ki-67 index was detected by IHC in all patients, which was evaluated and determined by two experienced pathologists. The yellow deposition in the nucleus is Ki-67 positive cells, and the expression rate of Ki-67 is the percentage of Ki-67 positive cells in the total number of tumor cells. The fraction of proliferating cells was based on a count of at least 500 tumor cells. The Ki-67 values were expressed as the percentage of positive cells in each case. Cases with >15% positive nuclei were classified as high Ki-67 expression, and those with <15% were classified as low Ki-67 expression.

RESULT

Of 979 subjects identified in the study, histopathologically confirmed cases of breast cancer patients data were analysed. Out of which (n=335) patients went under hormonal investigations. Tumors were separated into: ER+PR+, ER+PR-, ER+PR+HER2+, HER2 enriched andER-PR-HER2+.

Chart 1. Current treatment options in breast cancer are available at our centre.

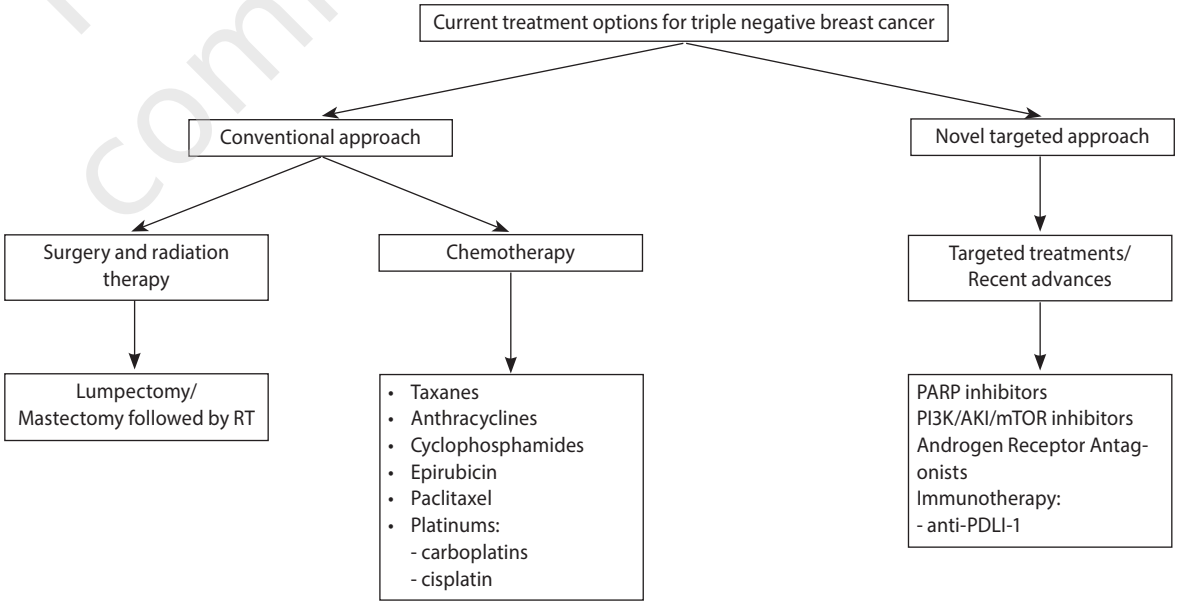


Chart 1 demonstrated the availability of treatment modalities at our centre for breast cancer and subtypes including TNBC patients. As per NCCN and ASCO guidelines treatment offered to the patients. Conventional and novel targeted approaches were the main treatment options including lumpectomy, mastectomy followed by RT. A recent advance in the management of BC is

Table 1 illustrated the numbers of patients in each subtypes. Luminal A (n=57; 29.62%), Luminal B (n=31; 17.01%), HER2 luminal (n=49; 14.63%), HER2 enriched (n=63; 18.81%) and TNBC (n=135; 40.3%). Patients with ER/PR+ (positive) status have 13.6% higher survival rate than those with ER/PR-. The overall survival for ER/PR positive is 72.1% compared to 58.5% of ER/PR negative. Triple neg-

Table 1. Subtypes of breast cancer.

Luminal A subtype	n	Luminal B subtype	n	HER2 subtype	n	TNBC subtype	n
ER+PR+HER2-	57	ER+PR-	31	Luminal HER2 ER+PR+HER2+	49	ER-PR-HER2-	135
				HER2 enriched ER-PR-HER2+	63		

n – numbers of patients.

atives cases were highest in numbers (n=135). Out of 335 cases of breast cancer hormonal status was studied in 173 (51.64%). women were postmenopausal and 162 (48.65%) were premenopausal. The ages of the study subjects ranged from 21 to 90 years. The median age was 50 years. Variables were taken as involvement of sites, stage and pathology of the disease, imaging modalities, treatment administered, follow ups and survival status.

Age wise analysis in table 2 illustrated the prevalence of hormonal status. Of 335 cases 76 (22.68%) patients were in old age groups (61–90). In middle age groups (41–60) a total of 169 (50.44%) cases were treated and 83 (25.07%) patients in younger age groups of 21–40 years. Highest numbers of hormonal receptor cases (50.44%) were recorded in middle age groups in this study.

Table 2. Age wise analysis of study subjects.

Age group (years)	Case (n)	ER +ve, number (%)	PR +ve number (%)	Both ER, PR +ve, number (%)	Both ER, PR -ve number (%)	HER2 -ve number (%)	HER2 +ve number (%)	Triple +ve number (%)	Triple -ve number (%)
21–30	31	9 (29.03)	7 (22.58)	11(35.48)	9 (29.02)	7 (22.58)	10 (32.25)	4 (12.90)	7 (22.58)
31–40	53	10 (18.86)	4 (7.54)	5 (9.43)	4 (7.54)	9 (16.98)	5 (9.43)	2 (3.77)	14 (26.41)
41–50	96	18 (18.75)	11(11.45)	15 (15.62)	17 (17.70)	9 (9.39)	7 (7.29)	2 (2.08)	21(21.87)
51–60	73	12 (16.43)	10 13.69)	13 (17.80)	17 (23.28)	9 (12.32)	0 (0)	1 (1.36)	11(15.06)
61–70	48	4 (8.33)	4 (8.33)	3 (6.25)	6 (12.5)	15(31.25)	8 (16.66)	0 (0)	8 (16.66)
71–80	17	3 (17.64)	2 (11.76)	3 (17.64)	1 (5.88)	4 (23.52)	3 (17.64)	1 (5.88)	1 (5.88)
81–90	11	3 (27.27)	2 (18.18)	1 (9.09)	1 (9.09)	1 (9.09)	1 (9.09)	1 (9.09)	1(9.09)

Table 3. Patient and tumor characteristics of overall breast cancer.

Total sample size	335	
Variables/ Parameters	Frequency	p – Value
Anatomical Site Involved		
Right	186 (55.52%)	P <0.0003
Left	131 (39.1%)	
Bilateral	18 (5.37%)	
ECOG criteria		
0	24 (7.16%)	P=0.0325
1	37 (11.04%)	
2	89 (26.56%)	
3	148 (44.17%)	
4	37 (11.04%)	
Staging as per AJCC		
Stage I	29 (8.65%)	P=0.0252
Stage IIA	23 (6.86%)	
Stage IIB	47 (14.02%)	
Stage IIIA	57 (17.01%)	
Stage IIIB	78 (23.28%)	
Stage IIIC	70 (20.89%)	
Stage IV	31 (9.25%)	
Pathology		
Histopathology/IHC	335	

ER+PR+HER2-	57 (17.01%)	P <0.0152
ER+PR-	31 (9.25%)	
ER+PR+HER2+	49 (14.62%)	
ER-PR-HER2+	63 (18.8%)	
ER-PR-HER2-	135 (40.29%)	
Imaging Modalities		
Mammography	331 (98.8%)	P=0.0152
CT/PET CT	263 (78.5%)	
MRI & USG Breast	41 (12.23%)	
Treatment		
Surgery	28 (8.35%)	P=0.0524
Chemotherapy	49 (14.62%)	
Immuno & targeted therapy	49 (14.62%)	
Surgery + Chemo	113 (33.73%)	
Surgery + Chemo + RT	52 (15.52%)	
CT + RT	2 (0.59%)	
Follow-up		
Patients currently on f/u	326 (97.31%)	P <0.0073
3 Monthly	43 (13.19%)	
6 Monthly	71 (21.77%)	
Annually	133 (40.79%)	
Twice in a year	79 (24.23%)	
Response evaluation (RECIST 1.1)		
Stable Disease	57 (17.48%)	P=0.0852
Partial Response	40 (12.26%)	
Complete Response	94 (28.83%)	
Disease Progression	135 (41.41%)	
Survival status		
On F/u	326 (97.31%)	P <0.0042
Lost to F/u	9 (2.76%)	
NED (No Evidence of Disease)	39 (11.96%)	
OS	117 (34.92%)	
Alive	236 (72.39%)	
Expired/Mortality	46 (14.11%)	
Recurrent/ residual disease	44 (13.49%)	

Tables 1, 2 and 3 show subtypes of breast cancer, age and association with other variables. Most of the tumours were located on right side (n=186; 55.52%), on the left there was 131 (39.1%) cases and bilateral was 18 (5.37%); not significant (P<0.0003). As per ECOG performance status criteria 148 (44.17%) patients were on scale 3, 89 (26.56%) – on scale 2 and 24, 37, and 37 – on scale 0, 1 and 4 respectively statistical value (P=0.325) in our study. Remarkable finding in this study Ki-67 level was increased in >50% cases of TNBCs which was significant (P=0.05). As per AJCC staging criteria 205 (61.19%) cases were in stage (locally advanced breast cancer). The value was significant (P=0.05). In early breast cancer and advanced stage of BC cases 29% and 9% were respectively. Imaging scan had a significant role in the diagnosis of disease in the preliminary, during the treatment and routine follow-up. Mammography was done in 98.1%, PET CT/ CT in 78.5% and MRI & USG breast in 12.23% patients (P <0.0524). In majority of the cases (n=113; 33.73%) combination treatment modalities (surgery followed by chemotherapy) given. In HER2 subtype (triple positive) (n=49; 14.62%) patients were treated with targeted therapy (trastuzumab) with standard and recommended dose of 440 mg/cycle. TNBC and mTNBC patients were treated with 1st, 2nd and 3rd line of chemotherapy with poor response and Ki-67 levels were >50% in TNBC and mTNBC cases. Clinical examination and RECIST 1.1 was done for response evaluation. Immunotherapy administered in (n=49; 14.62%) cases (P <0.0073). This literature reported 326 patients were on 6, 12 and 24 months follow-ups including alive patients 236 (72.39%), mortality rate were 46 (14.11%), NED in 39 (11.96%) patients, OS rate were 117 (34.92%) with statistical value (P <0.0042).

DISCUSSION

This literature reported in >50% cases of BC involvement were seen in right side and the left side in 39% cases with highest numbers of TNBC and mTNBC in the same anatomical site. Abdou et al. had mentioned in BC was more common in left side and

Figure 1. Demonstrated the status of FISH in HER2 +ve.

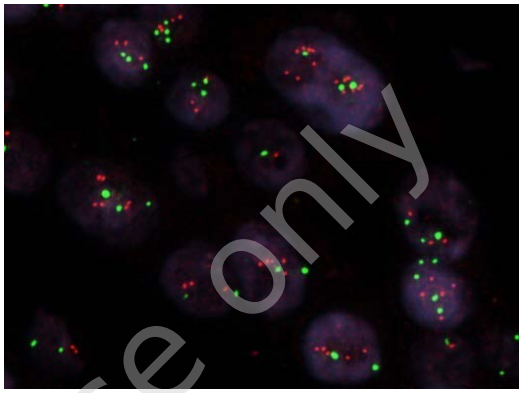
FISH MARKER		RESULT (POSITIVE)
HER2		
Total number of cell scored	100	
Total number of HER2 signals	470	
Total number of CEP17 signals	220	
Average HER2 signals/cells	4.7	
Computed ration	2.14	
Probe used: HER2: CEP17 dual color probe. HER2 orange, CEP17: green		**Figure 1**
Evaluation of this specimen shows an abnormal hybridization pattern. These findings are indicative that the patient is eligible for anti HER2 therapy (trastuzumab). HER2 gene amplification is seen 18–20% of invasive breast cancers.		

Figure 2. Demonstrated the status of FISH in HER2 -Ve.

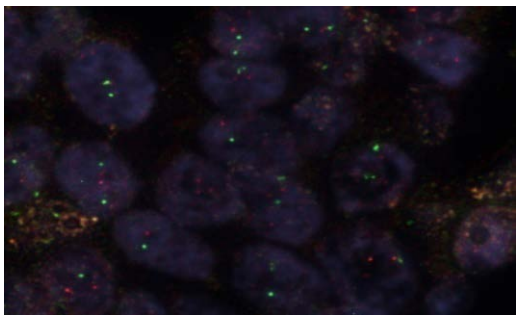
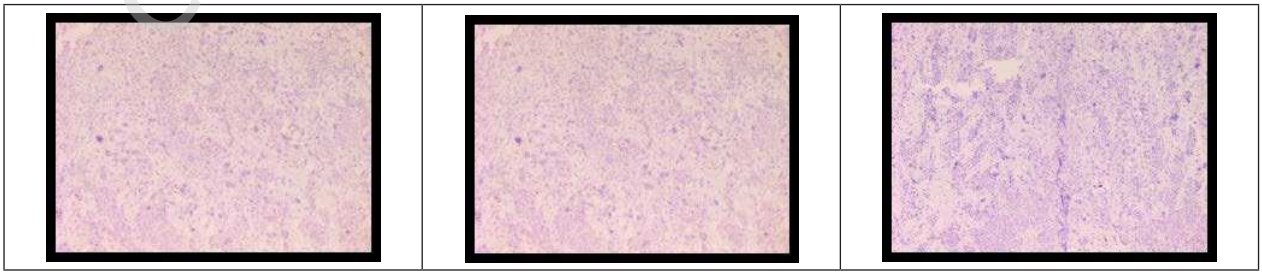
FISH MARKER HER2		RESULT NEGATIVE
Total number of cell scored	100	
Total number of HER2 signals	252	
Total number of CEP17 signals	170	
Average HER2 signals/cells	2.52	
Computed ration	1.48	
Probe used: HER2/CEP17 dual color probe. HER2: orange CEP17: green		**Figure 2**
Evaluation of this specimen shows a normal hybridization pattern. These findings are indicative that the patient is not eligible for anti HER2 therapy (trastuzumab). It has been recognized as a poor prognosis indicator in early breast cancer.		

Figure 3. Microscopic view of early and advanced stage of a TNBC case in 43-year-old women.



associated with aggressive biology and worsen outcomes [13]. The median age of time of diagnosis of BC was 40 and 50 years in India however 60 and 70 years in the western world as per literature published Alkabban et al. [14]. Early-onset BC is considered to be very aggressive with poor prognosis than late-onset BC [15]. Approximately 8% of Indian women presented with stage 1 disease in our literature and in Indian context too. Leong et al. demonstrated only 1% women presented with stage 1 disease in the United States. Leong et al. also presented in their paper about 10% of women in the United States present with stage IV disease, in India this number is approximately 6% to 24%, with approximately 29% to 52% of Indian women presenting at stage III [16]. This paper also revealed 75% of cases presented in locally advanced breast cancer stage. In the present study TNBC cases were in highest numbers 40%, ER-PR-HER2+ 18%, ER+PR-HER2-17%, ER-PR-HER2+ 14% and triple positive 9% only of all subtypes. Sandhu et al. showed in India TNBC prevalence was estimated at 31%, much higher than the Western prevalence of 12% to 17% [17]. Our literature revealed surgery alone was performed in 8% of early breast cancer cases and remain the mainstay. Surgery fol-

lowed by chemo in 33%, surgery, chemotherapy and radiation – 15%, and immunotherapy and targeted therapy administered in 14% cases of BC. Goldhirsch et al. also recommended the surgery in early breast cancer which ranges from lumpectomy to modified radical mastectomy [18]. The strong correlation seen in TNBC patients with high levels of Ki-67 in >50% cases and had a worse prognosis. Wang et al. also reported that high expression of Ki-67 (>40%) significantly correlated with worse prognosis in TNBC patients [19]. Only 2% patients were lost to follow up in this paper. Rigorous telephonic follow-up done. Swaminathan et al. reported follow-up in our country is a big challenge, factors like distances, stigma of cancer, dependency on family members, financial stress [20]. Overall survival reported in the literature was 34%, mortality rate reported 14%, recurrence in 13% and 72% patients were alive.

CONCLUSION

Hormonal receptor investigation was found to be playing a significant role in survival of the breast cancer patients. More focused research will help clarify underlying determinants of TNBC and for addressing the burden of breast cancer mortality in India. Need of the hour is to conduct larger multicentre trials in underprivileged cities of India where TNBC and other subtypes are more prevalent. Ki-67 could be considered possible prognostic factor. High expression of Ki-67 reflected lower survival rates.

STRENGTHS AND LIMITATIONS OF THE LITERATURE

Our study had several strengths. It was long term retrospective study with a large sample size that included patients at a tertiary cancer centre in eastern part of India. All tumors/tissues were processed and subtyped by a team of experienced pathologists and technicians.

We had to perform germ line sequencing for variants that predisposed to BC i.e. BRCA1 and BRCA2 pathogen variants but could not performed. Due to lack of high end laboratory facilities. However at our centre foot falls of BC cases are in large numbers. For FISH confirmation central lab facilities were borne. Through grant proposals our center is trying to get funds to enhance our laboratory facilities to conduct FISH and sequencing.

MESSAGE FOR FUNDING AGENCIES:

The projected population of Bihar is over 13 crores and has very limited molecular facilities in state capital Patna. As per data from cancer registries heavy foot falls of cancer patients are moving out of the city to seek better treatment. In these settings prognostic markers, FISH, genetic testing and counseling, validation of prospective clinical trials are the need of the hour. Pharmaceutical players and government (central and state) funding bodies must identify the potential sites in tier ⅔ cities of India to conduct larger multicentre trials in breast cancer.

ORCID:

Vijay Pratap Singh – https://orcid.org/0000-0002-8999-1992
Arun Kumar Jha – https://orcid.org/0000-0003-4081-4271
Chandan Kumar Singh – https://orcid.org/0000-0002-2387-1437
Akash Kumar Singh Singh – https://orcid.org/0000-0002-1202-8743
Avinash Pandey – https://orcid.org/0000-0002-1604-8717
Anita Kumari – https://orcid.org/0009-0007-5667-0192
Vishal Mohan Singh – https://orcid.org/0009-0004-7360-0696
Amrita Rakesh – https://orcid.org/0000-0002-6849-5818
Anadita Jha – https://orcid.org/0009-0006-6523-9444

References

1. Executive Summary. Census of India 2011. https://censusindia.gov.in/2011-prov-results/paper2/data_files/Bihar/4-ex-summery.pdf.
2. Ferlay J, Soerjomataram I, Dikshit R et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015; 136(5): E359-86. http://doi.org/10.1002/ijc.29210.
3. Brierley JD, Gospodarowicz MK, Wittekind C. TNM classification of malignant tumors. John Wiley & Sons, 2016.
4. Cao SS, Lu CT. Recent perspectives of breast cancer prognosis and predictive factors Oncol Lett. 2016; 12: 3674-8.
5. Giuliano AE, Connolly JL, Edge SB et al. Breast Cancer-Major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin. 2017; 67(4): 290-303. http://doi.org/10.3322/caac.21393.
6. Sung H, Ferlay J, Siegel RL et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71: 209-49.

7. Ferlay J, Ervik M, Lam F et al. Global cancer observatory: Cancer today. Lyon, France: International Agency for Research on Cancer; 2020. https://gco.iarc.fr/today/en.
8. DeSantis C, Siegel R, Bandi P et al. Breast cancer statistics, 2011. CA Cancer J Clin. 2011; 61(6): 409-18. http://doi.org/10.3322/caac.20134.
9. Schlatter RP, Matte U, Polanczyk CA et al. Costs of genetic testing: Supporting Brazilian Public Policies for the incorporating of molecular diagnostic technologies. Genet Mol Biol. 2015; 38(3): 332-7. http://doi.org/10.1590/S1415-475738320140204.
10. Torre LA, Siegel RL, Ward EM et al. Global cancer incidence and mortality rates and trends – an update. Cancer Epidemiol Biomark Prev. 2016; 25(1): 16–27. https://doi.org/10.1158/1055-9965.
11. Boyle P. Triple-negative breast cancer: Epidemiological considerations and recommendations. Ann Oncol. 2012; 23(suppl 6): vi7-12.
12. Brewster AM, Chavez-MacGregor M, Brown P. Epidemiology, biology, and treatment of triple-negative breast cancer in women of African ancestry. Lancet Oncol. 2014; 15: e625-34.
13. Abdou Y, Gupta M, Asaoka M et al. Left sided breast cancer is associated with aggressive biology and worse outcomes than right sided breast cancer. Sci Rep. 2022; 12(1): 13377. http://doi.org/10.1038/s41598-022-16749-4.
14. Alkabban FM, Ferguson T. Breast Cancer. In: StatPearls. StatPearls Publishing, Treasure Island (FL) 2023. https://www.ncbi.nlm.nih.gov/books/NBK482286/.
15. Anders CK, Hsu DS, Broadwater G et al. Young age at diagnosis correlates with worse prognosis and defines a subset of breast cancers with shared patterns of gene expression. J Clin Oncol. 2008; 26: 3324-30.
16. Leong SP, Shen ZZ, Liu TJ et al. Is breast cancer the same disease in Asian and Western countries? World J Surg. 2010; 34: 2308-24.
17. Sandhu GS, Erqou S, Patterson H et al. Prevalence of triple-negative breast cancer in India: Systematic review and meta-analysis. J Glob Oncol. 2016; 2: 412-21.
18. Goldhirsch A, Winer EP, Coates AS et al. Personalizing the treatment of women with early breast cancer: Highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol. 2013; 24: 2206-23.
19. Wang W, Wu J, Zhang P et al. Prognostic and predictive value of Ki-67 in triple-negative breast cancer. Oncotarget. 2016; 7(21): 31079-87. http://doi.org/10.18632/oncotarget.9075.
20. Swaminathan R, Rama R, Shanta V. Lack of active follow-up of cancer patients in Chennai, India: implications for population-based survival estimates. Bull. World Health Organ. 2008; 86: 509-15.

Authors' contributions:
Conceptualized the project. Principal investigators of the project. From planning to execution of the project played a very crucial role in designing of the study – Vijay Pratap Singh; Akash Kumar Singh. Literature writing, computer simulations, statistical analysis, material preparations, data collections etc. – Arun Kumar Jha. Result analysis – Chandan Kumar Singh. Clinical part and literature reviews – Anita Kumari; Vishal Mohan Singh; Avinash Pandey; Amrita Rakesh; Ajeet Kumar Singh; Rahul Kumar Choudhary. Data analysis in result section – Anandita Jha. Analysis of slides and blocks of breast cancer patients – Pranab Kumar Verma; Manish Jaipuriyar; Shambhwi Sharma; Sanjay Kumar.

Conflict of interests:
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The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed with the content of the manuscript as written. The paper complies with the Helsinki Declaration, EU Directives and harmonized requirements for biomedical journals.