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Prognosis of breast cancer patients and survival rate related with Ki-67 proliferation index: multicenter retrospective study

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Abstract

Breast cancer commonest cancer of urban Indian women and the second commonest in the rural women. It has different epidemio-clinical characteristics compared with European countries. The purpose of this study is to investigate prognosis and survival rate of breast cancer patients related with Ki-67 proliferation index in multicenter through retrospective study. We reviewed all patient's data in registration center of Medical Oncology Departments, in the period 2016-2020. Data has been collected on 760 breast cancer patients. Our resulted data showed most prevalent comorbidities associated with breast cancer were cardiovascular diseases, diabetes and anemia. Ki-67 index >28% mostly associated with early age female, lymph node involvement (p=0.001), advanced tumor grade (p=0.00012) and risk of relapse (p=0.004). Survival rate was decreased in patients with (ER, PR, Her_2 negative (triple negative breast cancer.

Keywords: Ki-67 index, Breast cancer, Retrospective study

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Introduction

Breast cancer is the most frequently diagnosed cancer and the most frequent cause for cancer-related deaths in women worldwide. Globally, breast cancer accounted for 2.08 million out of 18.08 million new cancer cases (incidence rate of 11.6%) and 626,679 out of 9.55 million cancer-related deaths (6.6% of all cancer-related deaths) in 2019 [1]. In India, breast cancer has surpassed cancers of the cervix and the oral cavity to be the most common cancer and the leading cause of cancer deaths [2]. The lowest breast cancer incidence is reported from Far Eastern and South-East Asian countries [3]. In the developing countries of Asia, the health care burden on account of breast cancer has been steadily mounting. It is expected that in the coming decades, these countries would account for majority of new breast cancer patients diagnosed globally [4]. Over 100,000 new breast cancer patients are estimated to be diagnosed annually in India [5]. As per the ICMR-PBCR data, breast cancer is the commonest cancer among women in urban registries of Delhi, Mumbai, Ahmedabad, Calcutta, and Trivandrum where it constitutes > 30% of all cancers in females [6].

In the rural PBCR of Barshi, breast cancer is the second commonest cancer in women after cancer of the uterine cervix [7]. The age standardized incidence rates (AARs) range from 6.2 to 39.5 per 100,000 Indian women. The AARs vary from region, ethnicity, religion, with the highest incidence reported at 48.3 per 100,000 women in the Parsi community of Mumbai [8]. In addition, Ki-67 index which is a

predictive and diagnostic biomarker that plays a significant 75 prognostic role [9]. Almost a third of all breast cancer patients are believed to have familial disease pattern, and some 5% are believed to be hereditary, with the BRCA1 and BRCA2 gene mutations having been identified as the major genetic causes. In an Indian study on 226 breast cancer patients, 20.7% had a positive family history [10]. On the contrary, numerous other studies have reported a low rate of familial pattern of breast cancer in Indian patients. This is particularly interesting given the relatively young age of Indian breast cancer patients. At SGPGIMS Lucknow, only about 5% of all patients managed had a definite family history of breast and/or ovarian cancer in first degree relatives, similar to the figures available from other Indian centers [11]. Genetic screening/diagnosis is not routinely performed in most Indian center due to paucity of funds and facilities.

As a result, there is scarce data on the genetic composition and BRCA1/2 mutations in Indian patients. The available studies hint at a rather low incidence of BRCA mutations. In most populations, 6–10% of patients with breast cancer have mutation in BRCA gene irrespective of their family history [12]. Though there are no robust figures, various Indian studies have reported BRCA mutations in 9–25% of familial breast cancer cases [13]. The study demonstrated 3 novel BRCA1 mutations including a founder Ashkenazi Jewish BRCA1 mutation in Indian breast cancer patients [14]. The literature data showed that comorbidities negatively impacts overall breast cancer prognosis [15-20].

The purpose of this study is to investigate prognosis and survival rate of breast cancer patients related with Ki-67 proliferation index in multicenter through retrospective study.

Material and methods

We reviewed all patient's data in registration of multi-center of Medical Oncology Departments, in the period 2016-2020. Data has been collected on 760 breast cancer patients. The study was conducted according to the declaration of Helsinki and with the approval of the biomedical ethics committee of Indian Council of Medical Research, New Delhi. Several clinico-pathological parameters were investigated in this study including: age and delay at diagnosis (time between symptoms and final diagnosis of breast cancer), age at menarche, parity, age at first delivery, breastfeeding, oral contraceptive use, menopausal status, breast density, body mass index and comorbidities. Furthermore, consanguinity and data including information on family history of breast and ovarian cancers and other malignancies were also recorded. All patients had a biopsy with complete immunohistochemical evaluation before the initiation of any systemic therapy. TNM classification was based on reported clinical evaluation.

In addition, clinico-pathological parameters have been collected including tumor size, lymph node involvement, Scarff-Bloom-Richardson SBR grade, hormone receptors and HER2 status, Ki-67 index, treatment type, relapse, outcome. Patients with metastatic disease were excluded. Ki-67 (Clone MIB-1 (DAKO) dilution 1/100) was used for the automated immunohistochemical technique. The assessment procedure was manually performed on 10 fields using high magnification (x400). All the pathologists of the department were used to the estimation and evaluation of the ki-67 index. In order to determine if

there are special epidemiological and/or clinico-pathological differences between familial and sporadic breast cancer cases, we classified our cohort into two subgroups.

The selection of familial cases was based on several criteria mainly the family history of breast and ovarian cancers and the age at diagnosis; patients were selected if at least one of the following criteria was fulfilled: (1) Presence of at least two related first or second-degree breast cancer cases, (2) Breast cancer in young patients aged less than 36 years [21]. Presence of at least two cases of breast or ovarian cancer, regardless of age [22] triple negative breast cancer subtype. Secondly, in order to study the correlation of Ki-67 index with the clinicopathological features and its survival prediction in the luminal breast cancer group, we used several Ki-67 cut-off points (14%, 20%, 30% and 50%). Authors evaluated these cut-offs based on previously published data [23]. Ki67 is already well studied in the literature as a continuous variable. Intrinsic subtype classification into Luminal A, Luminal B and Triple negative was based on immuno-histochemical criteria.

Statistical Analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA). Quantitative variables were analyzed by Student's t test. The differences were determined by Chi-square test or a Fisher exact test.

Result

A total of 760 patients with breast cancer have been included in this study were diagnosed by histopathology. Among breast cancer patients that participated in the current study, 59.3% were premenopausal, 3.2% of patients were nulliparous and 3.4% were pregnant. Mean age at menarche was 13.6 years. Mean age at first childbirth was 21.4 years. 66.6% have received oral contraception and 13.3% of patients have never breastfed. According to the Breast Imaging-reporting and Data System (BI-RADS) classification, 63% of patients had dense breasts. Obesity defined by BMI ≥30kg/m^2 was seen in 25% of cases. In additions, most prevalent comorbidities associated with breast cancer were cardiovascular diseases (59.6%) and diabetes (13.5%). Familial cases represented 9.6% of breast cancer patients. The family history of ovarian cancer was observed in 7% of patients.

The mean age at diagnosis of breast cancer was 51.4 years (ranging from 33 to 77 years) and 7.6% of patients were ≤35 years. Mean clinical tumor size at diagnosis was 25.6 mm. Locally advanced tumors (T3, T4) were seen in 35.8% of patients, 33.4% of cases is multifocal and 76% of patients were diagnosed with lymph node positive disease. The median Ki-67 value was 49% (range, 1-90%), HER2+ (16.5%) and distant metastases at diagnosis were observed in 35.8% of patients. (8% of patients had tumorectomy and (71 %) had mastectomy while Neoadjuvant chemotherapy (CT) was performed in 124 cases (14%) and (21%) treated by adjuvant chemotherapy.

There was a significant difference in the distribution of age at diagnosis among familial and sporadic breast cancer cases (mean 42.46 vs 50.63 years) (Table 1). Indeed, familial breast cancer cases were significantly younger than sporadic patients (p=6.078). Furthermore, familial breast cancer patients were more likely to be premenopausal (p=0.001). A delay in diagnosis was observed in sporadic cases

(9.47 months) when compared to familial cases (6.19 months) but no significant difference between the two groups was observed. However, a statistically significant difference was observed regarding intraductal component (p=0.027) and a significantly high tumor stage (T3) was noted among familial cases (14.29%, p=0.015) (Table 1).

Variables	Familial cases *n*=112	Sporadic case *n*=648	P value
Mean age at diagnosis (years)	46.41	55.43	3.07
Mean diagnostic delay (months)	5.43	8.97	0.187
Menopausal status			
Premenopausal	91/140	230/391	0.001
Postmenopausal	47/1140	225/391	

Discussion

Variability in Ki-67 results is attributed in part to pre-analytical components including antigen retrieval, fixations, storage and staining techniques, with additional concerns of epitope loss. Efforts to standardize by exactly following the strict guidelines for identifying MIB-1-positive nuclei including steps of where and how to count them also showed no improvement. Inter observer variability of MIB-1 labelling index in breast cancer was found to be more problematic than expected by the authors [24].

The problem is compounded by the lack of clearly defined criteria for scoring/evaluation as well as cut-offs for Ki-67 interpretation. The general method has been average score across the sections, but some studies have focused on hotspot and compared the predictive value of both techniques [25]. Hottest spot predicted clinical outcome better than the average score across the sections in patients with HR+/HER2− cancers, as reported by the authors [26]. They attributed the results to the hottest spots, being the most biologically active part of the tumor which drives the outcome of the disease. It has also been postulated that the hottest spot may reflect the area, where the fixation condition is most suitable for Ki-67 staining [27]. Assessment at the hottest spot is also more convenient and relatively free from issues of area selection as in assessment across the slide. The cut-offs for Ki-67 have also been controversial ranging from 10 to 25 per cent and have been arbitrary. The mean age at diagnosis of breast cancer patients was 48.7 years [28].

This result is similar to those described in previous studies on breast cancer by other studies and different from that reported in patients from Western countries [29]. Inflammatory and triple-negative breast tumors were found among 5.35% and 15.5% of patients, respectively. These proportions of aggressive breast cancer forms are different from those described in previous studies (TNBC=22.5%, IBC=7-10%) [30] and could be considered as an update of latest data on these breast cancer forms in Indian population [31]. Furthermore, our results were different to those described in several other populations, such as Western and Sub-Saharan Africa where TNBC accounts for 27% to 61% of cases. For IBC, it represents 2% of breast cancer in Europe and USA [32], lower than that reported in North

Africa (5-10%). Furthermore, a delayed diagnosis for up to 6 months after first detection of a breast mass has been observed among 39% of patients [33].

Large mean tumor size, high proportions of T3 and T4 stages, lymph nodes involvement and patients diagnosed with distant metastases were observed. All these characteristics are considered as factors associated with late diagnosis of breast cancer [34]. These results showed that despite large awareness campaigns running in India, breast cancer is still shrouded in secrecy and is still considered a taboo for several socio-cultural reasons. In our study, luminal B cancer was more prevalent (46.27%) than luminal A (28%). This result is in discordance with previous Indian studies [35] which could be explained by the heterogeneity of breast cancer even in different cohorts drawn from the same country. The Inclusion of HER2 positive cases in luminal B groups could also be an explanation [36]. In addition, our results are in discordance with previous studies conducted in several countries all over the world including Japan and the USA [37]. Although, other studies conducted in Italy found luminal B subtype more common than luminal. This variation in the distribution of breast cancer molecular subtypes could be explained by the fact that different cut offs value of Ki-67 index are used for the stratification of luminal breast cancer groups across the world which may lead to misclassification bias [37]. The optimal cut off obtained by receiver operating characteristic (ROC) curve analysis for RT-qPCR in this study was 22.23 per cent, unlike 5.68 optimal cut-off point, using X-tile programme cut-off point for Ki-67 by RT-PCR [38]. The application of RT-qPCR has been reported with mixed results in the literature in their comparative study of RT-PCR HER2 versus IHC and fluorescence *in situ* hybridization (FISH) reported suboptimal performance of RT-PCR to IHC in terms of discriminative ability and clinical benefit [39].

Our results demonstrated that there was a statistically significant difference between the 2 groups (Ki-67≤20%) and (Ki-67>20%) regarding a set of parameters [40]. Tumor with Ki-67>20% showed the poorest prognosis; early age at onset, advanced tumors grade, positive node involvement, high risk of relapse (p20%) was also more frequently associated with HR-negative and HER2-positive tumors [40]. This result was in agreement with studies who reported that a higher Ki-67 index significantly correlated with HER2-positive breast tumors [42] and negatively correlated with HR positivity [41]. Additional analyses performed in the present study demonstrated that Ki-67 index predicted survival with a cut off value of 30% in the overall luminal breast cancer group and 50% in ≥4pN+ studied tumors. Previous report 290 demonstrated that a Ki-67 index with a cut-off (≥20%) is significantly correlated with poorer prognosis and early recurrence, particularly in luminal A type tumor [43].

Therefore, our study is first to evaluate Ki67 association with clinico-pathological features and outcome among Indian breast cancer patients and significant associations were observed. According to these results high levels above 30% could be used to classify tumors into Luminal B, but the data does not confirm that a Ki67 below 30% can be interpreted as luminal A. Furthermore, due to the lack of external validation study, caution should be used when implementing this cut-off as an independent marker; all other clinico-pathological parameters should also be considered when evaluating the prognosis of any given patient.

Conclusion

Based on these results, we conclude that Ki-67 expression is significantly associated poor prognosis of breast cancer patients and survival rate. We did not observe a significant correlation with other clinicopathological parameters included in the study.

Conflict of Interest

The authors declare that there is no conflict of interest.

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