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Assessment of Proliferation Index and Pathological Features as Prognostic Potential of Breast Cancer in Ethiopia

Endegena Abebe[1]*, Wondyefraw Mekonen[2], Daniel Seifu[2], Yonas Bekurtsion[2], Abebe Bekele[2] and Eva Johanna Kantelhardt[3]

[1]*Hawassa University College of Medicine and Health Sciences, Ethiopia*
[2]*School of Medicine, Addis Ababa University, Addis Ababa, Ethiopia*
[3]*Department of Bioinformatics, Martin Luther University, Halle, Germany*

Abstract

Background: Analysis of proliferation status in breast cancer can be associated with tumor aggressiveness. Uncontrolled proliferation has been accepted as a distinct hallmark of cancer and act as an important determinant of cancer outcome. Recently measuring proliferation used as predictive potential and crucial element of treatment decision in patients with breast cancer.

Aim: We therefore aimed to assess the frequency proliferation rate by using mitotic count and Ki-67 index to associate with other prognostic markers.

Methods: A prospective study of 197 newly diagnosed breast cancer tissues from women received surgery as initial management in three different hospital in Addis Ababa, Ethiopia from January 2013 to December 2015 were included in the study. Histology slides were evaluated for the histological type, grade (by modified Nottingham grade score). Mitotic count quantified as number of mitoses per 10 high power fields (HPF) at the tumor periphery. Less than and seven mitoses per 10 fields scored 1 point, 8-16 scored 2 points, and more than 17 scored 3 points. Immunohistochemical staining of Ki-67 was performed and the proliferation rate was expressed as percentage of stained tumor cell nuclei. Associations of the Ki67 index with other prognostic factors were evaluated both as continuous and categorical variables.

Results: The Mean ± SD mitotic activity index was 15.7 ± 10.6 while the median was 14/10 HPF. Mitotic count was found 0-7 mitoses per 10 HPF in 42/197 (21.3%) cases, between 8 and 14 mitoses per 10 HPF in 74/197 (37.6%) cases and >15 in 81/197 (41.1%) cases. Ductal type BC was associated with high mitotic count than lobular and other histological BC types which is statistically significant (p=0.009). High mitotic count was significantly associated with aggressive features of the primary tumors (negative hormonal receptor (ER- and PR-) p<0.001 and Negative Her-2/neu patients) p=0.01. The Mean ± SD Ki-67 expression level of the study participant was 24.9 ± 19.1 while the median expression level was 15% (range: 3–85%). Women age <35 yrs were found high mean value than women age > 60 yrs of old. The mean value of G2 and G3 tumor were higher than G1 tumor and it is statistically significant (p <0.001). The Ki-67 mean value was found lower in Hormone receptor posetive (ER +, PR +) than hormone receptor negative (ER-, PR-). The deference was statistically significant (p=0.001) and (p=0.005). While Her-2 posetive tumor found higher mean Ki-67 value than Her-2 negative tumor and also the difference was statistically significant (p=0.001).

Conclusion: Clinical utility of Mitotic count and Ki-67 in combination with other prognostic markers can better predict breast cancer outcome. Therefore we suggest that Ki-67 index should be added to treatment plan when considering adjuvant chemotherapy and prognostic stratification.

Keywords: Mitotic count; Ki 67 index-breast cancer; Prognostic potential; Chemotherapy; Hormone receptor

Introduction

Breast cancer is complex disease, comprising distinctive histological patterns. It has also shown divergent nature with regards to its clinical course, response to treatment, and prognostic outcomes. Thus, it's difficult to determine the biological behaviour and prognosis of breast cancer based on the assessment of a single factor [1]. A number of tumour and patient-related factors can be identified in order to understand the clinical behaviour of the newly diagnosed tumour to determine prognosis and survival [2]. The substantial fact on breast cancer in Ethiopia is like other African countries in its presentation; as it predominantly affects the younger population, frequently presents as higher histological grades and in advanced clinical stages [3].

Traditional factors known to predict the behaviour of breast cancer worldwide include tumour size, lymph node involvement, and distant metastasis more over in low and middle income countries they are also used to decide whether adjuvant systemic treatment is indicated [4,5]. Breast cancer is hormone-dependent tumour. Estrogen (ER) and progesterone (PR) play important roles in the growth and differentiation of breast cancers making them important prognostic markers [2]. Human epithelial receptor 2 (HER-2), a proto-oncogene also known as ErbB2-neu, is also considered to be closely associated with occurrence and development of breast cancer [2,6]. Different expression patterns of ER, PR and HER-2 have been identified, making knowledge of the receptor content of breast carcinoma essential in planning the management of disease. It is well established that ER/PR and HER-2/neu are the most powerful prognostic factors in deciding on treatment [7]. In Ethiopia, receptor status assessment for breast cancer patients is routinely unavailable. Few research reported the majority of

***Corresponding author:** Endegena Abebe, Professor, Hawassa University College of Medicine and Health Sciences, Ethiopia, Tel: +251911407062, E-mail: endgena.abebe@aau.edu.et

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patients in Ethiopia are ER-positive rather than ER-negative. However, tamoxifen has been prescribed based on evidence suggesting that ER/PR-positivity rates in Ethiopia are the same as in Western countries [8,9]. The overall effect of these changes is an increased role for tumour biology in clinical evaluation. However, identification of more other prognostic factors are needed to define low or average/high risk subgroups [10].

Carcinogenesis is a multistage process initiated by disturbed and uncontrolled proliferation of cells. The growth of malignant tumours is highly variable and this probably reflects their clinical course, however proliferation is a key feature of progression of tumour [11,12]. Cell cycle kinetics plays a vital role in tumor behaviors also in diagnosis, prognosis, monitoring and surveillance of BC in clinical practice [11,13]. Proliferative potential, beside it's a prognostic indicator in solid tumor and it has predictive ability to guide treatment but also claimed to explain the biological differences in the tumor [11].

Proliferative capacity have been quantified in many methods to assess the aggressiveness in the progression of cancer. The traditional mitotic count is still used to grade a variety of tumors to represent tumour cell proliferation [12]. Mitotic count is the number of mitoses per high power field (x 400 magnification) can determined on hematoxylin and eosin stained sections of tumours, and therefore, a routine part of histopathological evaluation. The major advantage is that histopathologic diagnosis and mitotic count/index can be determined at the same time, making it the most convenient method of evaluating cell proliferation. Mitotic count has shortcoming on standardization. Despite that mitotic count was a stronger predictor of survival than tumour size, lymphatic invasion or skin invasion in the report before two decades Clayton, [14] reported a study of 378 node-negative BCs and found the clinical implications. Elzagheid in 2006 [15] also reported on Caucasians, and proved that mitotic count is the best prognosticator of survival in BC particularly in lymph node negative patients. Boder [16] reported the proliferative indices did not show statistically significant differences of survival in LN- patients. Patients with more than 4.5 mitotic figures per 10 HPFs had a 2.8-fold increase in the risk of death.

Other method used to assess cell proliferation is the immunohistochemical indication of the Ki-67 antigen, which is a nuclear protein synthesized in the active phases of the cell cycle [13]. Ki-67 binding as an objective measurement of cell proliferation. Cells that showed specific nuclear staining were scored as positive and the Ki67 labelling index was expressed as the percentage of the total number of tumour cells that stain positive; this equates to the growth fraction of the tumour [13,14]. Higher grade cancers have higher Ki67 indices - one study found mean scores of 9% in grade I tumours, 14% in grade II and 26% in grade III [17]. Various studies have shown correlations between Ki67 and disease-free and overall survival, with an increased risk of recurrence in tumours with a high Ki67 [9,18]. Ki-67 become part of routine biomarker profile along with hormone receptor status and Her-2 to assists the clinicians to provide optimum treatment to breast cancer patients. In systematic review by Stuart-Harris [19] reported its role as predictive in both the antihormonal therapy and chemotherapy for the efficacy of the treatment. Patients with tumors that have a very high level of proliferation have a better response to chemotherapy [20]. Furthermore this marker could help select patients who are not capable to benefit from chemotherapy, such as those with Her/2-negative and hormone receptor- positive tumors with low proliferation [21]. Although there is no consensus on optimal cut-off point, it was stated that both in uni-and multivariate analyses the Ki67 index had prognostic information in cut-off index used varied between 0-30% [11] the current study was carried out to observe the frequency of proliferative activity and their distribution in invasive breast carcinoma in relation to predictive clinicopathological factors of Ethiopian women.

Methodology

A prospective analysis of 197 patient diagnosed as invasive breast cancer and treated by modified radical mastectomy or with wide local excision and axillary clearance, at three different Hospital in Addis Ababa were included in this study. Demographic data and disease related information including clinical presentation data were accrued. Formalin fixed paraffin embedded tumour tissue was used for the study. Five micron sections were stained with hematoxylin and eosin stain and reviewed by the experienced pathologist in order to classify according to the WHO classification. Routine mitotic count were determined using microscopes with a 400x magnification, a 40x objective and a field area of 159 μm^2. Mitoses were counted in 10 consecutive high power fields according to the criteria proposed by Dutra [21] Immunohistochemical staining was quantified after the sections were dewaxed with freshly prepared xylene for two minute then descending concentrations (100, 96 and 70%) of ethanol for three minute each the slides were washed in distilled water. Endogenous peroxidase activity was blocked in order to reduce background staining in freshly prepared 3% H_2O_2 before proper target retrieval. Slides were immersed into staining dish containing Antigen Retrieval Solution. We proceeded to heat-induced epitope retrieval method. Pre heat the steam cooker with retrieval solution (citrate buffer) pH 9 till 95°C at 750 W. The slides were placed in the cooker for 5 minute then allow the cooker to cool for 20 minute prior to opening. The slides were transferred to room temperature, washed with tween buffer and put the slides in humidity chamber. Further slides were incubated in IHC blocking buffer (Zytomed systems GMBH, Berlin, Germany) for 15 min to prevent nonspecific binding of antibodies. The IHC blocking agent was then drained and slides were incubated with the primary antibody for 1 h in a humidity chamber. After rinsing the primary antibody twice using tween wash buffer for 3 min in each, the slides were incubated in secondary antibody labelled with 3, 3 diaminobenzidine (DAB) substrate HRP (Zytomed systems GMBH, Berlin, Germany) for 1 h in the same chamber. Detection of labelled secondary ABC (Zytomed systems GMBH, Berlin, Germany) according to manufacturer's instructions. The sections were counter stained using haematoxylin, dehydrated using ethyl alcohol, cleared using xylene, and mounted in Entallen then examined under light microscope. All sections were performed at the same time and submitted to standard methods. The recombinant mouse anti-Ki-67 monoclonal antibody [clone MSK018; Monoclonal Antibody to Estrogen Receptor (ER), Immunostaining for PR was done using Mouse, Monoclonal Antibody to Progesterone Receptor and Monoclonal Antibody to c-erbB-2 Protein (Her-2/neu) (Zytomed Systems GmBH Berlin, Germany), Known Positive and negative cases were used as external controls. In our study, ER and PR status was considered positive if >1% of the cells were positively stained for the respective biomarker. Her-2 status was considered positive for all 3+ tumors and negative for 0, 1+, and 2+ tumors. The Ki-67 score was defined as the percentage of positively stained cells among the total number of malignant cells scored. The numbers of stained cancer cell nuclei were scored in 300 cancer cells and the ratios of stained to total cells expressed as percentage (0-100%) regardless of staining intensity were defined as Ki67 indices [22]. Besides evaluating Ki-67 as continuous variable, we calculated the frequencies of tumors

Criteria	N	%
Age		
≤ 35	63	32.0
36-60	106	53.8
>60	28	14.2
Menstrual status		
Perimenopause	111	56.7
Postmenopausal	86	43.7
Stage		
I	6	3
II	54	27.4
III	113	57.4
IV	24	12.2
Lymph node involvement		
No	70	35.5
N1	127	64.5
Histology		
Ductal	123	79.2
Lobular	74	8.1
Others	25	12.7
Tumor size		
≤ 2 cm	11	4.1
>2 cm	189	95.9
Histological grade		
G1	34	17.3
G2	91	46.2
G3	72	36.5

Table 1: Distributions of clinicopathological features among study participants (n=197).

Characteristics	Mitotic count N (%)			N (%)	p value
	0-7	8-14	>15		
Age					
Mean ± SD	41.4 ± 12.1	45.6 ± 13.7	45.4 ± 11.84	44.64 ± 13.31	0.2
Menstrual status, n (%)					
Premenopausal	25 (59.5)	44 (59.5)	44 (44.3)	113(57.4)	0.7
Postmenopausal	17 (40.5)	30 (40.5)	37 (45.7)	84 (42.6)	
Lymph mode status, n (%)					
Lo	18 (42.9)	33 (44.6)	23 (28.4)	74 (37.6)	0.08
L1	24 (57.1)	41 (55.4)	58 (71.6)	123 (62.4)	
Stage, n (%)					
I	2 (4.8)	2 (2.7)	2(2.5)	6 (3)	0.3
II	9 (21.4)	25 (33.4)	20 (24.7)	54 (27.4)	
III	24 (57.1)	43 (58.1)	46 (56.8)	113 (57.4)	
IV	7 (16.7)	4 (5.4)	13(16)	24 (12.2)	
Tumor size, n (%)					
<2 cm	5 (11.9)	6 (8.1)	6 (7.4)	17 (8.6)	0.5
2- 5 cm	29 (69)	51 (68.9)	50 (61.7)	130 (66)	
>5 cm	8 (19)	17 (23)	25 (30.9)	50 (25.4)	
Histology, n (%)					
Ductal	27 (64.3)	60 (81.1)	69 (85.2)	156 (79.2)	0.009*
Lobular	9 (21.4)	4 (5.4)	3 (3.7)	16 (8.1)	
Others	6 (14.3)	10 (13.5)	9 (11.5)	25 (12.7)	
ER, n (%)					
Negative	4 (9.5)	11 (14.9)	48 (59.3)	63 (32)	0.001*
Positive	38 (90.5)	63 (85.1)	33 (40.7)	134 (68)	
PR, n (%)					
Negative	8 (19.0)	18 (24.3)	52 (64.2)	78 (39.6)	0.001*
Positive	34 (81.0)	56 (75.7)	29 (35.8)	119 (60.4)	
HER-2, n (%)					
Negative	35 (83.3)	45 (60.8)	62 (76.5)	142 (72.1)	0.01*
Positive	7 (16.7)	29 (39.2)	19 (23.5)	55 (27.9)	

Table 2: The association between mitotic activity index group and clinicopathological features of BC (n=197).

with three levels of risk category Ki67 index; low (<15%), intermediate (16-30%), and high (>30%) [11,23]. Moreover, we used of quartiles to determine the impact of Ki-67 was based on the mean value to set a cut-off between minor/major 14% (<12, 12–14, 15–29, ≥ 30%) the St Gallen recommendations [14,15]. The study was approved by the ethical review committee of the Addis Ababa University College of Health Sciences and the patients gave informed consent. Data obtained were analyzed using the Statistical Package for the Social Sciences version 20 statistical package (SPSS) Incorporated, Chicago, Illinois, USA. Descriptive values obtained from the data were presented as mean ± SD, median (minimum-maximum), and number and frequency as percentages. ANOVA were used to test differences between the groups. The relationships between the categorical characteristics of the groups were examined by chi-square test. In all the analyses, p-values below 0.05 were regarded as significant.

Results

Clinicopathological features of the study

A total of 197 female breast carcinoma were included in the study. Out of study participants, 106/197 (53.8%) were between age of 36-60 years, only 28/197 (14.2%) were age >60 years while the rest of the

Character	N	Ki- index (Mean ± SD)	95% CI		p-value
			lower	upper	
Age group, n					
<35	63	26.77 ± 20.63	21.10	32.44	0.14
36-60	106	25.44 ± 18.5	21.87	29.01	
>60	28	18.39 ± 11.05	14.10	22.68	
Histological grade, n					
G1	34	13.20 ± 10.15	9.66	16.74	<0.001*
G2	91	20.42 ± 12.1	17.9	22.95	
G3	72	35.98 ± 23.8	30.39	41.57	
Lymph node status, n					
N0	74	22.94 ± 18.1	18.68	27.19	0.27
N1	123	25.97 ± 19.78	22.47	29.47	
ER, n					
Positive	134	19.11 ± 13.53	16.79	21.41	<0.001*
Negative	63	37.11 ± 21.4	31.21	43.01	
PR, n					
Positive	119	21.76 ± 16.87	18.66	25.41	0.005*
Negative	78	29.60 ± 21.53	24.76	34.45	
HER-2, n					
Positive	55	37.46 ± 21.1	31.69	43.22	<0.001*
Negative	142	20.11 ± 16.09	17.45	22.77	
ER/PR status, n					
ER + PR+	100	20.69 ± 14.82	17.75	23.63	<0.001*
ER + PR-	33	14.48 ± 7.1	11.96	17.02	
ER- PR+	16	30.18. ± 26.24	16.20	44.18	
ER- PR-	48	38.93 ± 22.24	32.47	45.39	

Table 3: Differentiation of continuous Ki67 percentages by analysis of variance (ANOVA) and histopathological parameters (n=197).

Characteristics	Ki-67 category N (%)			N (%)	p value
	Lower<15%	Intermediate 16-30%	High >30%		
Age (Mean ± SD)	45.34 ± 14.4	43.93 ± 13.12	44.6 ± 11.84	44.64 ± 13.31	0.6
Lymph mode status, n (%)					
Absent	28 (35.9)	32 (43.8)	14 (30.4)	74 (37.6)	0.3
Involved	50 (64.1)	41 (56.2)	32 (69.6)	123 (62.4)	
Stage, n (%)					
I	3 (3.8)	2 (2.7)	1 (2.2)	6 (3)	0.2
II	21 (26.9)	20 (27.4)	13 (24.1)	54 (27.4)	
III	48 (61.5)	39 (53.4)	26 (56.5)	137 (57.4)	
IV	6 (7.7)	12 (16.4)	6 (13.0)	24 (12.2)	
Tumor size, n (%)					
<2 cm	9 (11.5)	3 (4.1)	5 (10.9)	17 (8.6)	0.07
2- 5 cm	45 (57.7)	57 (78.1)	28 (60.9)	130 (66)	
>5 cm	24 (30.8)	13 (17.8)	13 (28.3)	50 (25.4)	
Histological grade, n (%)					
G1	27 (34.6)	6 (8.2)	1 (2.2)	34 (17.3)	<0.001*
G2	33 (42.3)	51 (69.9)	7 (15.2)	91 (46.2)	
G3	18 (23.1)	16 (21.9)	38 (82.6)	72 (36.5)	

Table 4: Cross analysis between Ki-67 risk category and other tumor characteristics (n=197).

participants were age <35 years. Among study participants, 111/197 (56.7%) were pre-menopausal. Ductal type breast carcinomas were most common 123/197 (79.2%) and majority of the tumor were >2 cm at initial diagnosis 189/197 (95.9%). Additionally, stage three tumor was seen nearly in two third 113/197 (57.4%) of the study (Table 1). Study participants had more hormone receptor positive and Her/2 negative. ER positive (68%), PR positive (60.4%), and HER2 negative (72.1%) tumors (Figure 1).

Proliferation activity using mitotic activity index

Mitotic count was assessed in the tumours of this study by histologic examination. The threshold applied for the assessment of the mitotic component of the Nottingham histological grade was used. Mitotic count was found 0-7 mitoses per 10 HPF in 42 (21.3%) cases, between 8 and 14 mitoses per 10 HPF in 74 (37.6%) cases and >15 in 81 (41.1%) cases, the mean was 15.7 while the median 14/10 HPF (Figure 2).

Table 2 Illustrates younger patients have low mitotic index than intermediate and high mitotic index. Patients with lymph node involvement showed high mitotic count than lymph node negative. Ductal type BC was associated with high mitotic count than lobular and other histological BC types which is statistically significant (p=0.009). High mitotic count was significantly associated with aggressive features of the primary tumors (negative hormonal receptor (ER- and PR-) p<0.001 and Negative Her-2/neu patients) p=0.01.

Proliferation index assessment by IHC using Ki67 marker

Evaluation of absolute Ki 67 expression with correlation to clinical parameter: Ki67 values and their relation to the histopathologic parameters were examined using ANOVA, as shown in Table 3. The mean Ki-67-labeling index for all the patients was nearly 25% (Mean ± SD 24.86 ± 19.1), the median of Ki-67 expression level was 15% (range: 3–85%). The mean values were higher in women age <35 years old than women age between age of 36 and 60 years and women above age of 60 years which was not statistically significant (p=0.14). G1 tumors had Ki-67-labeling index of 13.20% that was near to the median value while, the mean value of G2 and G3 tumor were 20.42%, and 35.98% respectively and it is statistically significant (p<0.001). Concerning the nodal involvement, differences of Ki- 67 values were not more visible. Mean Ki67 in node-negative tumors was 22.94% whereas in node Positive were 25.97% which is not statistically significant (p=0.2). Interestingly, in terms of BC stage, stage I BC had a Ki-67 expression of 11.16% in contrast to the stage IV tumors which had a mean Ki-67

Characteristics	Ki-67 category N (%)			N	p value
	Low	intermediate	high		
ER, n (%)					
Negative	12 (19.0)	17 (27.0)	34 (54.0)	63	<0.001*
Positive	66 (49.3)	56 (41.8)	12 (9)	134	
PR, n (%)					
Negative	25 (32.1)	23 (29.5)	30 (38.5)	78	<0.001*
Positive	53 (44.5)	50 (42.0)	16 (13.4)	119	
HER-2, n (%)					
Negative	77 (54.2)	40 (28.2)	25 (17.6)	142	<0.001*
Positive	1 (1.8)	33 (60.0)	21 (45.7)	55	
Chi-Square test *significant (p<0.05)					

Table 5: Distribution of hormone receptor and HER-2status in proliferative (Ki67) risk category among study participants (n=197).

Characteristic		Ki-67 (%) 1st Quartile < 12%	Ki-67 (%) 2nd quartile 12–14%	Ki-67 (%) 3rd quartile 15–29%	Ki-67 (%) 4th quartile ≥ 30%	Total n (%)	p value
Age (Mean ± SD)		46.21 ± 15.45	44.78 ± 13.84	43.41 ± 12.59	44.87 ± 12.71	44.64 ± 13.3	0.83
Menstrual status	Pre	12 (42.9%)	27 (54%)	34 (64.2%)	38 (57.6%)	111(56.3%)	0.3
	Post	16 (57.1%)	23 (46%)	19 (35.8%)	28 (42.4%)	86 (43.7%)	
Tumor size	0-2 cm	1 (3.6%)	7 (14%)	0 (0%)	0 (%)	8 (4.1%)	<0.001**
	>2 cm	17 (96.4%)	43 (86%)	53 (100%)	66 (100%)	189 (95.9%)	
Nodal status	N0	11 (39.3%)	17 (34%)	21 (39.6%)	21 (31.8%)	70 (35.5%)	0.7
	N1	17 (60.7%)	33 (66%)	32 (60.4%)	45 (68.2%)	127 (64.5%)	
Stage	I	2 (7.1%)	1 (2%)	1(1.9%)	2 (3%)	6 (3%)	0.7
	II	8 (28.6%)	13 (26%)	13 (24.5%)	20 (30.3%)	54(27.7%)	
	III	17(60.7%)	31 (62%)	32 (60.4%)	33 (50%)	113 (57.4%)	
	IV	1 (3.6%)	5 (10%)	7 (13.2%)	11 (16.7%)	24 (12.2%)	
Histology grade	G1	16 (57.1%)	11 (22%)	5 (9.4%)	2 (3%)	34 (17.3%)	<0.001*
	G2	11 (39.3%)	21 (42%)	34(64.2%)	25 (37.9%)	91 (46.2%)	
	G3	1 (3.6%)	18 (36%)	14 (26.4%)	39 (59.1%)	72 (36.5%)	
ER	Pos	25 (89.3%)	38 (76%)	40 (75.5%)	31 (47.0%)	134 (68.0%)	<0.001*
	Neg	3 (10.7%)	12 (24%)	13 (24.5%)	35 (53.0%)	63 (32.0%)	
PR	Pos	23 (82.1%)	27 (54%)	33 (62.3%)	36 (54.5%)	119 (60.4%)	0.04*
	Neg	5 (17.9%)	23 (46%)	20 (37.3%)	30 (45.5%)	78 (39.6%)	
Her-2	Pos	0	1 (2%)	22 (41.5%)	32 (48.5%)	55 (27.9%)	<0.001*
	Neg	28 (100%)	49 (98%)	31 (58.5%)	34(51.5%)	142 (72.1%)	
* Statistically significant							

Table 6: Associations between Ki-67 quartiles and the histopathological parameters(n=197).

of 27.33%. The mean Ki-67 values in ER + and PR+ was 20 and 22% respectively. In receptor negative tumors, absolute Ki-67 values with a mean of Ki-67 of ER- and PR- was 37.11 and 29.6% respectively. The combinational expression of ER and PR are considered, four subgroups are recognized: double Hormone receptor Positive (HR+) ER+/PR+), single HR+ (ER+/PR- and ER-/PR+) and double HR- (ER-/PR-) the absolute Ki-67 values with a mean of Ki67 of 20.69% both in ER Positive and in PR Positive tumors while 38.93% was recorded in both receptor negative. Mean Ki67 of Her-2 Positive tumors was 37.6%, and 20.11% in Her-2 negative tumors.

Cross analysis between Ki67 risk category and clinicopathological features of BC: Ki67 was categorized into high (>30%), intermediate (16-30%) and low (<15%) levels of proliferating 39.6%, 37.1% and 23.4% of the tumor respectively (Figure 3). lymph node absent tumors shown low and intermediate proliferating as compared to tumors with lymph node involvement. Table 4 also demonstrates that the distribution of grade 1 tumors were only 2.2% in high proliferating category while the remaining cases were in low and intermediate proliferation category that is statistically significant (p<0.001). The ER, PR and Her-2status showed a significant association to cell proliferation (Table 5). Thus, ER (+) or PR (+) tumors showed low proliferation category. There was inverse relation between Ki67 index ER and PR positivity. Whereas direct relation was seen with Her-2 positivity, however high Ki67 (>30%) was associated with decreased Her-2 positivity as compared to intermediate Ki67 (16-30%).

Ki-67 quartiles and histopathological parameters

The distribution of 4th quartile Ki-67 was high in premenopausal patients, while postmenopausal patients were predisposed to to lower Ki-67 percentages. The frequency of 1st quartile expression of Ki-67 was seen in, tumor with <2 cm. In tumor >2 cm, the 4th quartiles Ki-67 expression were predominant. Concerning the nodal status, it was shown that in node negative tumors the first Ki-67 quartile was slightly higher than the 3rd and 4th quartile. The distribution of Ki-67 in relation to grading showed, in low-grade tumors (G1), the first and the second Ki-67 quartiles were predominant. The distribution of high-Ki-67 percentages in G1 tumors was only 3%. Conversely, high-grade tumors were associated with high-Ki-67 quartiles. Only 3.6% of G3 tumors were found in the 1st quartile in contrast to 59% in the 4th quartile. Estrogen receptor (ER) positive distribution were progressively decrease from low to high quartile while in ER negative the quartile distribution progressively increasing from 1st to 4th quartile. The same phenomena was observed in progesterone receptor (PR), the distribution. In terms of Her-2, high-Ki-67 quartiles were found in tumors with Her-2 positive none of the cases were in the first and second quartile. Most of Her-2 negative was correlated with low Ki-67 values than high quartile.

Discussion

BC aggressiveness appears to be directly related to the proliferation percentage of cancer cells. Different approaches are used to quantity cell proliferation including mitotic counts classical method to expressing the mitotic activity, estimation of the fraction of cells in S-phase of the cell cycle and immunohistochemistry of proliferation-associated antigens [11]. Mitotic index is the classical technique to assess cell proliferation on routinely stained histological slides but it is time consuming and has a relatively low reproducibility. A close correlation between the mitotic count and some of the clinicopathological features was shown in the BC patients. However, the biological mechanisms responsible for these mitotic count variations in the tumor cells remain to be disclosed,

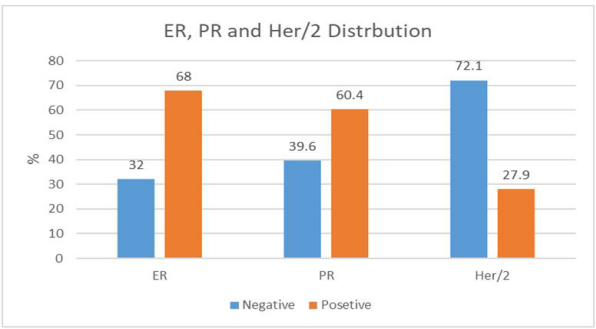

Figure 1: ER, PR and Her/2 distribution among study participants.

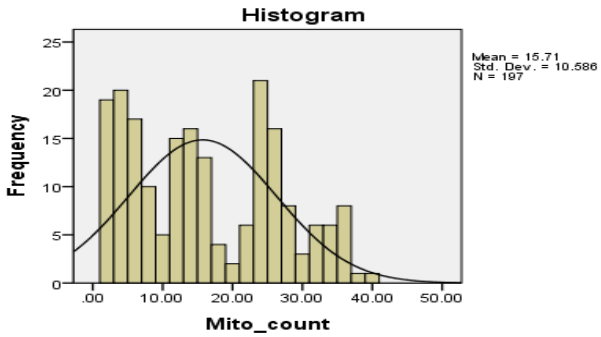

Figure 2: Distribution of mitotic activity index values in BC cases.

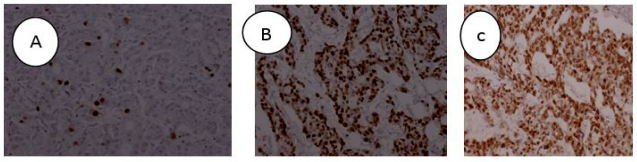

Figure 3: Photomicrograph representative 40x pictures of immunohistochemical KI-67 staining (A) low, (B) intermediate and (C) high.

although certain mutations in growth-regulating genes may contribute to the high mitotic activity seen [24]. Counting mitotic figures by light microscopy is still a relatively rapid and low cost method for the estimation of tumor cell proliferation. In addition, some investigators have found mitotic figure content to be a good prognostic indicator in patients with breast carcinoma [21]. In this work (Table 5), proliferative difference was not observed between pre and postmenopausal patient groups. Unlike data published by Boder. In the present study, there was a significant correlation between high mitotic figure count per 10 HPF and histological type, ER, PR and Her-2 negative. Our work was supported by other studies [24,25]. We believe our work has to validate by survival studies but it could be a contributing marker to indicate chemotherapy in combination with receptor status. Comparison between studies was difficult due to differences in the reporting of mitotic counts, the microscopes field diameter and area adjustment. Previous work by Baak, reported survival was less likely in cases with a score 3 (>17 mitosis/10 HPF) compared to those with a score 1(0-7 mitosis/10HPF) by using mitotic activity index reporting [26,27]. In our study more aggressive features of BC significantly associated with mitotic index score 3(>17 mitosis/10 HPF). Due to its simplicity we suggest to report the exact number of mitosis parallel to its score used for histological grade.

Due to lack of consensus regarding cut off values of Ki67, In our study, we used the cut-off level 14% was based on the work of Cheang who subtyped 357 patients with invasive breast cancer by gene expression profiling and together with IHC determination of hormone receptor status [27]. The review by Yerushalmi also concluded the Ki-67 level above 10-14% was defined as high risk group in terms of prognosis [28].

It can be also used as continuous variables, based on the proportion of positive tumor cells (0-100%) irrespective to staining intensity. The Ki67 labelling index important for selecting the addition of chemotherapy to endocrine therapy in hormone receptor-positive breast cancer, and classified and clustered tumors as risk levels as low, intermediate, and highly proliferating according to the value of Ki67 labelling index of less than or equal to 15%, 16-30%, and more than 30%, respectively [23,29]. Ki-67 as a continuous variable, significant association was found between mean Ki67 index and histologic grade, ER, PR, Her-2 and BC subtype. The mean value of the Ki67 index in our study was 24.8% (± 19.1%), median of 15%. Accordingly, with respect to age distribution at diagnosis, the mean and median values for Ki67 indices for the ages between < 35 years of age is higher than the rest. Although many studies establish that Ki-67 proliferation index is higher in very young patients, [11,23] we were not able to determine a similar result in our study. The possible explanation for this is due to the uneven distribution number of patients.

Histological grading has been one of the most commonly used parameters for therapy decision-making for a long time. Histological grade can clearly subdivide tumors into low and high risks groups (grade I vs. grade III) in terms of outcomes. However, about 40-50% of BCs are classified as grade II with a less well-defined risk. The use of Ki-67 index in a grade I population could be particularly useful to sub-classify them. We found in our study the mean value of 14.47 for grade I tumors which is near to the cut value while High grade tumors showed 29.5 near to the value of high proliferative index. In line with data from Pakistan by Haroon [30] demonstrated grade III tumor had high proliferation index while grade I tumor had mean value of 17.29. In our study we found the mean value of 21.83 in Grade II cases and data from Pakistan also demonstrated 26.97. Therefore the use of Ki-67 index in a grade II population could be particularly useful to pay attention to them. Lymph node involvement also seen in our study with a higher absolute Ki-67 index values is also comparable to other studies [9,30,31]. Likewise advanced tumor stages, larger tumor and nodal involvement were seen in higher Ki-67 quartiles indicating more aggressive behavior our study was comparable to a work done in Bavaria, Germany from the clinical cancer registry data [9]. This result strengthens the assumption of Ki- 67 as predictive potential and sparks the importance of Ki-67 in routine clinical work to enhance the most commonly used parameters for therapy decision-making for a long time.

This study demonstrated the relationship between Ki67, ER and PR status. In the current study, A significant inverse relationship between the Ki67 index and hormone receptor positivity was observed, ER (-) tumors more often had higher Ki67 indices as compared to ER (+) tumors. With the higher rates of ER positivity shown in the lowest proliferating tumors which is similarly reported previous work from Asian and Western patients [3,31,32]. A similar correlation was seen in tumors with PR negative, classified as highly proliferative group. A study conducted in Sudan, failed to reveal any significant association of Ki67 with hormone receptors [33]. A study carried out in Iranian population showed significant correlation between PR and Ki67 but correlation with ER was not found [34]. Ki67 expression was reported to be significantly associated with a favorable prognosis following neoadjuvant hormone replacement therapy and lower recurrence rate [34-36]. Our study suggests that importance of measuring Ki67 and comparing hormone receptor levels help to determine prognosis, and as a guide to adjuvant chemotherapy.

In this study Table 6, the relationship between Ki67 expression levels and Her-2status was assessed, and found that Ki67 expression was higher in Her-2 Positive patients. Therefore Ki-67 may differentiate Her-2 positive tumors with good and poor prognosis. Our finding was similar to reports of several studies which demonstrated Her-2 Positive tend to have higher proliferation rates [37-39]. In the other hand, a report from Japan described that Ki67 expression was not correlated to Her-2 status [28]. Another study reported 10% and 20% of Her-2 posetive Vietnamese and Swedish patients respectively had low Ki-67 expression that may doubt the proliferation rate to classify the aggressive cancers but in our study only 1% of Her-2 positive found in low expression category (< 15%) and there was no report of Her-2 posetive in 1st quartile Ki-67 expression by using 14%. However, there is no agreement on cutoff value for Ki-67 in Her 2 Positive BC that will define good and poor prognosis groups that needs investigation. This should be a subject of further research [40-42].

The study demonstrated that the Ki-67 showed an association with the common histopathologic parameters by using absolute expression, categories and Ki-67 quartiles the effect was clearly seen in the association between Ki-67 and staging, grading, ER, PR and Her 2 receptor. This result reinforces the assumption of Ki- 67 as predictive and prognostic marker. Similarly advanced tumor stages and higher nodal status were associated with higher Ki-67 quartiles indicating that the more aggressive the tumor is the higher is the percentage of cells positively stained for Ki-67.

Conclusion

The assessment of proliferation is one of the major factors for the treatment decisions in BC patients. The determination of mitotic index or count is an inexpensive, fast and reproducible way of assessing proliferation in routine practice. But, Ki-67 is a more convenient method for assessing the proliferation, Ki-67 may reflect the aggressive behavior of BC and predict the degree of risk which may determine the appropriate therapy. The study demonstrated that both Mitotic count and Ki-67 showed an association with the common histopathologic parameters. Moreover, Ki-67 as absolute expression and Ki-67 categories was clearly seen in the association with grading, ER, PR and Her 2 receptor. Our data showed that Mitotic count and Ki-67 may be used in routine clinical work to enhance the most commonly used parameters for decision-making and treatment follow-up of BC patients. The level of Ki 67 can be included in the pool of prognostic markers like tumor size, nodal status, histopathological grade and hormonal receptors.

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

EA participated in the study design, data collection and coordinated data management, Laboratory work and statistical analysis. EA also wrote the initial manuscript. WM and DS participated in study design and data management supervising the work. YB Laboratory work and acquisition of data. AB participated in study design, data collection, data management and manuscript preparation. EJK contributed in supervising the work. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by Addis Ababa University college of Medicine and health Sciences Ethics Committee. Protocol # IRB 012/2015. Also National Ethics and research committee. Protocol NERC 310/006/2015. Written and informed consent was obtained from every patient.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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