Asian Journal of Biochemistry, Genetics and Molecular Biology

4(2): 60-67, 2020; Article no.AJBGMB.58737
ISSN: 2582-3698

The Potential Role of Ki-67 Index in the Prognosis of Breast Cancer and Its Relation to Molecular Subtypes in Egyptian Females with Breast Cancer

F. Z. Mohammed[1], Lamis Gamal[2*], Mohamed Farouk Mosa[3] and Mohamed Ibraheim Aref[3]

[1]Department of Biochemistry, Faculty of Science, Zagazig University, Egypt.
[2]Department of Chemistry- Biochemistry, Faculty of Science, Port Said University, Egypt.
[3]Department of Clinical Pathology, Faculty of Medicine, Al-Azhar University, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJBGMB/2020/v4i230104
Editor(s):
(1) Dr. Arulselvan Palanisamy, Muthayammal Centre for Advanced Research (MCAR), Muthayammal College of Arts and Science, India.
Reviewers:
(1) Ying Yin, Affiliated Zhongda Hospital of Southeast University, China.
(2) Shailja Chatterjee, MMCDSR, M. M. University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/58737

Original Research Article

Received 01 May 2020
Accepted 07 July 2020
Published 13 July 2020

ABSTRACT

Background: Breast cancer (BC) is a well-documented major cause of female morbidity and mortality worldwide. Ongoing research era is focusing on the establishment of diagnostic and prognostic markers, helping for early pick up of the cases, proper prognosis evaluation and clarifying reliable treatment strategy.
Aim of the Study: This study aimed to evaluate the role of Ki-67 as prognostic marker for breast cancer in Egyptian females population.
Patients and Methods: 120 BC patients and 30 age and BMI matching health controls are the subjects of the study, Ki-67 index values were investigated by immunohistochemistry that was performed on 5-lm slides of formalin-fixed and paraffin-embedded archival tumor tissue (core needle biopsy samples). Antigen retrieval was performed in a micro-oven in citrate buffer pH 6 for 20 minutes. Ki-67–stained slides were captured digitally at a hot spot at 3200 magnification. The Ki-67 labeling index was measured using digital image analysis software. Image analysis was performed

**Corresponding author: E-mail: Maifawzy2007@yahoo.com;*

by an experienced pathologist. Ki-67 index values were correlated with the clinicopathologic aspects of the BC patients.
Results: Our study showed that Ki-67 index values revealed gradual increase with disease severity and correlated with poor prognosis aspects.
Conclusion: Ki-67 index values are shown to be associated with breast cancer prognosis, supporting their role as prognostic biomarkers.

Keywords: Breast cancer; Ki-67; prognosis; molecular subtypes.

1. INTRODUCTION

Breast cancer is highly heterogeneous in terms of its etiology and pathological characteristics, some cases are showing slow growth with excellent prognosis, whereas other cases are taking a highly aggressive clinical course. Much effort is made on the scientific, economical, and organizational levels for better understanding of the eliciting factors, the molecular motivations for progression and the best effective, least hazardous intervention lines [1].

The use of breast cancer biomarkers has been widely applied, such as Ki-67 that is considered one of the most promising ones. Cell proliferation is a mainstay in determination of BC invasiveness, and it is intimately related to the disease poor prognosis. Accordingly, attention is paid to Ki-67 as a proliferation index. Ki-67 is a time saving, little manipulated and relatively cheap method which require as small tissue sample as could be obtained from fine-needle aspirations (FNA) [2].

Considering these facts, this study aimed to evaluate the role of Ki-67 as a prognostic biomarker in Egyptian females with BC.

2. PATIENTS AND METHODS

2.1 Patients

This is study was performed in Zagazig university hospital, Zagazig, Egypt, from January 2019 to January 2020. Zagazig university ethical committee approved the study.

The study groups:

- Group 1 (control): 30 healthy female subjects, age and BMI matching to the patient group. All showed normal screening mammogram with no family history of breast cancer, no history of breast mass, pain, abnormal discharge or breast skin changes.
- Group 2 (patients): 120 breast cancer patients that were, with recently pathologically proved breast cancer, of different disease stages (I, II, III and IV):

Stage I: T1N0M0.

Stage II: T0N1M0, T1N1M0, T2N0M0, T2N1M0 or T3N0M0.

Stage III: T0N2M0, T1N2M0, T2N2M0, T3N1M0, T3N2M0, T4N0M0, T4N1M0, T4N2M0 or any T N3M0.

Stage IV: any T any N M1.

Patients clinical and pathological data [lesion size (T), node status (N), presence or absence of metastasis (M), tumor grading,ER, PR and HER2 results] were retrived from patients medical records. Patients with smoking and or metabolic diseases as well as patients receiving hormone replacement therapy were excluded from the study.

The study subjects were classified according to their molecular subtype [3]:

1. Luminal A: ER and/or PR positive and HER2 negative.
2. Luminal B: ER and/or PR positive and HER2 positive.
3. Her2 positive (non-luminal): ER and PR negative and HER2 positive.
4. Triple negative: ER negative, PR negative and HER2 negative.

2.2 Methods

Ki-67 index assessment

- Immunohistochemistry was performed on 5-lm slides of formalin-fixed and paraffin-embedded archival tumor tissue.
- A micro-oven was used for performing antigen retrieval for 20 minutes in citrate buffer pH 6.

- The Ki-67 antibody was diluted 1:500, incubated in a TechMate 500 plus (Dako) for 25 minutes, and visualized with diaminobenzidine. (clone MIB1, Dako, Glostrup, Denmark).
- Immunohistochemistry for Ki-67 was performed on core needle biopsy samples.
- Immunohistochemistry for Ki-67 was repeated on the surgical resection specimen for all cases.
- Image Analysis: The slides stained with Ki-67 were captured at a 3200 magnification hot spot. The measured area was about 0.25 mm for the selected hot spot.
- The Ki-67 index values were measured by digital image analysis software (Tissue Studio 64 Dual, version 3.5, Munich, Germany) [4].

2.2.1 Image analysis

- This was performed by an experienced pathologist.
- For proper expressing selected area, the pathologist excluded the normal breast tissue. However, some contamination with stromal and lymphoid tissue could not be excluded.
- The interpreting pathologist scored at least 1000 cells. These cell numbers were scored in fields that were seen to be representative on an initial overview of the whole section.
- According to methodology of Arihiro et al. we distinguished normal cell elements from cancer cell nuclei on the image as following: "nuclei with little areas (32 lm2 gross area, which was decided by a mean nuclear area of 50 infiltrating lymphocytes, 31.7 lm2, and by mean nuclear area of 50 normal ductal cells, 31.1 lm2) and spindle shape (more than 0.5 oval rate) were considered as lymphocytes, normal ductal cells, and stromal cell nuclei, respectively, and were excluded" [5].

2.3 Statistical Analysis

MedCal_version 17.9.7 software was used for the analysis of the (MedCalc Software bib, Ostend, Belgium). Quantitave data were expressed as mean and standard deviation, while qualitative data were expressed as frequency and percentage. Nottingham prognostic index (NPI) values of the patients were calculated and interpreted [6]. Pearson tests were carried out for correlation tests. ROC curve analysis was done to estimate cutoff point for differentiation between healthy subjects and breast cancer patients.

3. RESULTS

Age and BMI (Table 1): Table 1 shows that there is no statistically significant difference in age and BMI among the different studied groups.

3.1 Histopathological Type and Tumor Grade

The most prevalent histolopathological type of BC (99 cases; 82.5%) was invasive ductal carcinoma (IDC). 9 cases (7.5 %) were invasive lobular carcinoma (ILC), 4 cases (3.34%) were mucinous carcinoma; 3 cases (2.5%) were medullary carcinoma, 3 cases (2.5%) were malignant phyllodes tumor and 2 cases (1.66%) were poorly differentiated carcinoma. Regarding to the tumor grade, 12 patients were of grade I (10%), 79 patients were of grade II (65.8%) and 29 patients were of grade III (24.2%).

Molecular subtype of breast cancer patients (Fig. 1).

Fig. 1 shows that the most prevalent molecular subtype of BC was Her2 positive type, with little smaller percent of the luminal B type and the least was luminal A type.

NPI (Table 2): Table 2 shows that most of the patients were of moderate prognosis, and the least were of excellent prognosis according to NPI.

Ki-67 index values (Tables 3, 4 and Fig. 2).

Table 3 shows that there was significant difference between stage I and stage II patients regarding to the Ki-67 index values, no significant difference was noted between stage II and stage III patients and high significant difference between the stages III and IV.

Table 4 shows that there were no significant difference between excellent and good prognosis groups regarding to the Ki-67 index values. Highly significant increase in this biomarkers as the prognosis get worse is evident.

Fig. 2 shows that there was significant difference in the Ki67 among the different molecular subtypes of the breast cancer, with the highest percent was noted in the triple negative type.

Table 1. Mean ± SD of women age and BMI among studied groups

Groups / Parameter	Control group (Group I) n =30	Breast cancer group (Group IIa) Stage I n=30	(Group IIb) Stage II n=30	(Group IIc) Stage III n=30	(Group IId) Stage IV n=30
Age (years)	48.3 + 9.7	50.1+ 12.4	49.3+9.9	48.9 +10.2	50.3+11.1
p		>0.05	>0.05	>0.05	>0.05
BMI (kg/m2)	31.5 + 6.1	30.4+7.6	30.8+6.6	31.3 + 6.4	29.2+ 11.4
p		>0.05	>0.05	>0.05	>0.05

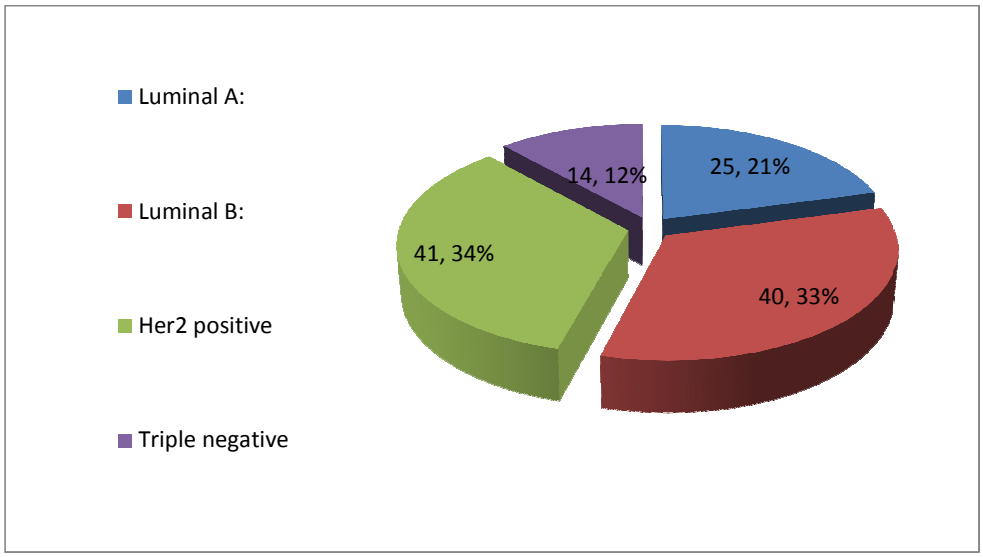

Fig. 1. Molecular subtypes of breast cancer patients

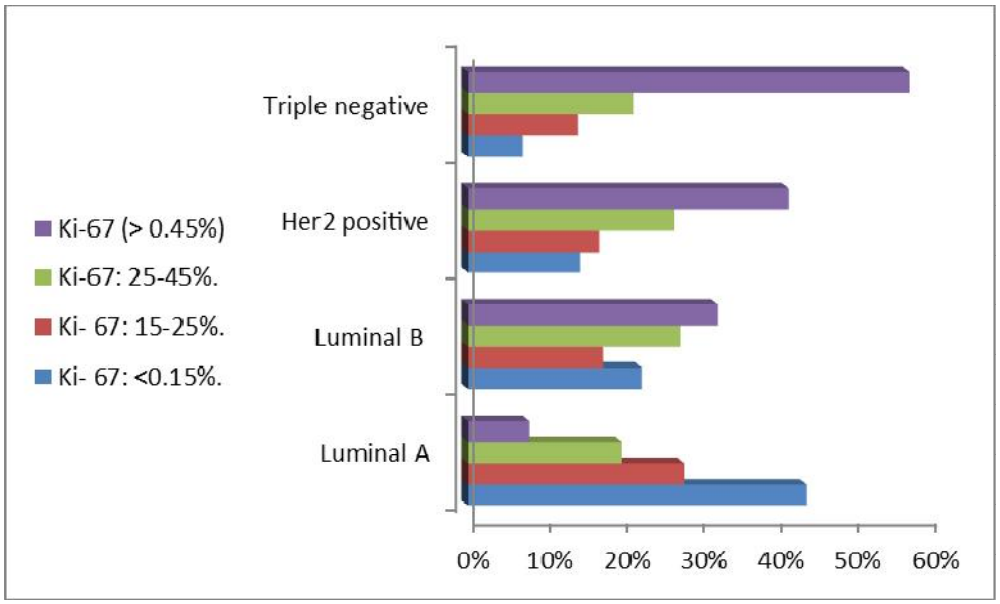

Fig. 2. Percent of different categories of Ki-67index values in the different molecular subtypes of the breast cancer patients (p<0.01)

Table 2. The breast cancer patients prognosis according to the NPI values

Patients prognosis according to NPI	N	Percentage
- Excellent prognosis.	2	1.67%
- Good prognosis.	19	15.83 %
- Moderate prognosis.	75	62.5%
- Poor prognosis.	24	20%
* Total	120	100%

Table 3. The mean values of Ki-67 index values in the groups of the study

Mean±SD	C	Stage I	Stage II	Stage III	Stage IV
KI-67(%)		24.7±12.7	27.5±15.7	28.7 ±17.9	44.6±27.5
p			<0.05	>0.05	<0.01

Table 4. Mean ± SD of different biomarkers in the breast cancer patients after their stratification according to the NPI values

Mean±SD	Excellent prognosis (n= 2)	Good prognosis (n= 19)	Moderate prognosis (n= 75)	Poor prognosis (n= 24)
KI-67(%)	14.3 ±6.44	16.7±6.3	27.3±11.5	39.7 ±18.6
p		>0.05	<0.01	<0.01

Pearson correlation testing of the Ki-67 index values with the clinicopathological characteristics of the patients is shown in Table 5. It shows that KI-67 index values revealed significant positive association with the tumor size, node status, tumor grade and NPI values.

The ROC curve analysis revealed that 22% Ki-67 cutoff value was reliable to differentiate subjects with excellent and good prognosis from ones with moderate prognosis, and 31% Ki-67 cutoff value was reliable to differentiate subjects with moderate prognosis from those with poor prognosis.

4. DISCUSSION

Breast cancer, like other cancers, occurs because of an interaction between an environmental (external) factor and a genetically susceptible host [7]. Normal cells cycles include division and stopping division. Cells become cancerous when they lose their ability to stop dividing, and thereafter, disorganization of the attached cells occurs. Also the normal apoptotic process organization is lost. All over the world, breast cancer is the commonest malignancy in females. It comprises 22.9% of malignant tumors in females [8].

Prognostic markers are essential for management plan of various cancers. Ki-67 index in breast cancer patients is recently considered as predictive and prognostic indicator for the disease [9]. However, its cutoff values is varied and not globally fixed.

At the present, in view of the obviously simple and economic methodology in Ki-67 testing, this has been commonly used worldwide for the BC patients prognostic evaluation [10]. Previous studies showed considerable testing for the correlation between Ki-67 index values and prognosis of BC [2,11].

Table 5. Correlations between the Ki-67 index values and different clinic-pathological parameters in the breast cancer patients

Variables	Ki-67	
	R	P
Age	0.12	>0.05
Tumor size	0.6	<0.01
Node status	0.68	<0.01
Tumor grade	0.63	<0.01
NPI values	0.61	<0.01

In this study, we assessed the BC patients Ki-67 values. There was significant difference between stage I and stage II patients, no significant difference between stage II and stage III patients and high significant difference between stage III and stage IV, who shows significant difference between them. Furthermore, we assessed the difference in Ki-67 levels after patients stratification according to the NPI categories into excellent, good, moderate and poor prognosis groups, our findings are supporting the

prognostic role of Ki-67, as there were significant differences among the good, moderate and poor prognosis groups.

Various studies have evaluated the Ki-67 index values variation among the breast cancer molecular subtypes. Soliman et al. concluded a high Ki-67 index (> 15%) in 34% & 60% of Her2 and triple negative breast cancer respectively [12]. On the other hand, we found an even high Ki-67 in these two subtypes of breast cancer; about 86.4% and 92.9% of Her2 and triple negative breast cancers respectively had Ki-67 > 15% in our study.

St. Gallen international expert consensus on primary therapy for early breast cancer 2013, defined surrogate clinicopathologic definitions of intrinsic breast cancer subtypes taken into account percentage of PR positivity (cutoff>20%) and Ki-67 index. There was a disagreement on the exact cutoffs for Ki-67 index. Although a cutoff value of 20% was proposed, especially for the adjuvant use of chemotherapy; however cutoff value of 14% beast correlated with gene expression definition of luminal A breast cancer [13].

We assessed the relation between Ki-67 levels and patients clinico-pathologic parameters, including patient age, tumor size, nodes status, the histopathological grade and NPI value. Ki-67 levels showed no significant correlation with the patient's age, while they showed significant correlation with tumor size, node staging, the histopathological grade and NPI values. It was reported that the Ki-67 high levels show significant association with poor prognosis, short survival, and high mortality rate [14,15,16]. None of the previous studies, to the best of our knowledge, has revealed that Ki-67 is significantly correlated with patient's age, which is in concordance with this study [14,17]. Few studies concluded the Ki-67 significant correlation with tumor histopathological grade [18,19,20]. This result indicates the intimately related behavior of the tumor grade and the Ki-67 status, both are related with active proliferation. However, further research is needed before conclusion of the relationship of Ki-67 and tumor grade [21]. Bouzubar et al results also were consistent with ours, as they found significant correlation between tumor histopathological grade and Ki-67 status, and no significant one between the age, the tumor size or the nodes staging and the Ki-67 levels [22]. In contrary to our results, Molino et al. concluded significant association with node staging, as they found that N0 tumors are likely to have a lower Ki-67 levels [23]. Also, some studies concluded that Ki-67 was significantly positively correlated with tumor size, in which, lower Ki-67 levels are associated with smaller tumors [24]. However, in other studies, there was no significant correlation between Ki-67 and tumor grade [14].

Inconsistency in determining cutoff values is evident; this may be partially secondary to lack of validation of the inter-laboratory results. Previous meta-analysis studies concluded 25% ki67 as cutoff value [25], other studies suggested 20% Ki-67 as cut off value of impact the poor prognosis in breast cancer [26,27]. Another meta-analysis showed that elevated Ki67 levels were correlated with worse survival [28]. Most recently, Zhu et al study concluded that patients with Ki-67 > 30% were correlated with poor survival [29].

ROC curve analysis in this study revealed that 22% Ki-67 cut off value was reliable to differentiate subjects with excellent and good prognosis from ones with moderate prognosis, and 31% Ki-67 cut off value was reliable to differentiate subjects with moderate prognosis from those with poor prognosis.

5. CONCLUSION

Ki-67 index values are shown to be associated with breast cancer prognosis, as they revealed gradual increase with disease severity, and they showed significant positive correlation with tumor size, tumor grade, node status and NPI values, supporting their role as prognostic biomarkers. 22% Ki-67 cut off value was reliable to differentiate subjects with excellent and good prognosis from ones with moderate prognosis, and 31% Ki-67 cut off value was reliable to differentiate subjects with moderate prognosis from those with poor prognosis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Baldassarre G, Belletti B. Molecular biology of breast tumors and prognosis. F1000 Research Journal. 2016;13(1):5-9.

2. Hashmi A, Hashmi K, Irfan M, Khan S, Edhi M, Ali J, Hashmi S, Asif H, Faridi N, Amir Khan A. Ki67 index in intrinsic breast cancer subtypes and its association with prognostic parameters. BMC Res Notes. 2019;12:605.
3. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ. Strategies for subtypes--dealing with the diversity of breast cancer: Highlights of the St. Gallen international expert Consensus on the primary therapy of early breast cancer. Ann. Oncol. 2011;22(8):1736–1747.
4. Baatz M, Zimmermann J, Blackmore G. Automated analysis and detailed quantification of biomedical images using Definiens Cognition Network Technology. Combinatorial Chemistry & High Throughput Screening. 2009;12(9):908–916.
5. Arihiro K, Oda M, Ohara M. Comparison of visual assessment and image analysis in the evaluation of Ki-67 expression and their prognostic significance in immune histochemically defined luminal breast carcinoma. Japanese Journal of Clinical Oncology. 2016;46(12):1081–1087.
6. Garner J. In the case of breast cancer, how can these be combined to give prognostic information? Questions for the MRCS vivas. London: Arnold. 2004;231.
7. Abdel-Aziz T, Azab N, Emara N, Odah M, El-deen IM. Study of BRCA2 gene mutations in Egyptian females with breast cancer. International Journal of Innovative Research in Science. 2015;4(2):283-288.
8. Saunders, Christobel, Sunil. Breast cancer, Oxford University Press. 2009; Chapter 13:978.
9. Kim Y, Seok JY, Hyun KY, Lee GH, Choi SC. Correlation of glasgow pronostic score or procalcitonin to clinical variables in patients with pretreatment lung cancer. Biomedical Science Letter. 2016;22:9-17.
10. Pathmanathan N, Balleine RL. Ki67 and Proliferation in Breast Cancer. J. Clin. Pathol. 2013;66:512–516.
11. Haroon S, Hashmi AA, Khurshid A, Kanpurwala MA, Mujtuba S, Malik B, Faridi N. Ki67 index in breast cancer: Correlation with other prognostic markers and potential in Pakistani patients. Asian Pac J Cancer Prev. 2013;14(7):4353–4358.
12. Soliman NA, Yussif SM. Ki67 as a prognostic marker according to breast cancer molecular subtype. Cancer Biol Med. 2016;66:2095–3941.
13. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, Senn HJ. Panel members: 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. Ann Oncol. 2013;24:2206–23.
14. Kermani TA, Kermani IA, Faham Z, Dolatkhah R. Ki-67 status in patients with primary breast cancer and its relationship with other prognostic factors. Biomed. Res. Ther. 2019;6(2):2986-2991.
15. Liu Y, Zhang X, Yu F, Liu J, Zhang M, Zhang S. Prognostic significance of Ki-67 expression before and after neoadjuvant chemotherapy in different biological breast cancer phenotypes. Zhonghua Zhong Liu Za Zhi. 2014;36(9):671–6.21.
16. Matsubara N, Mukai H, Fujii S, Wada N. Different prognostic significance of Ki-67 change between pre- and postneoadjuvant chemotherapy in various subtypes of breast cancer. Breast Cancer Res Treat. 2013;137(1):203–12.
17. Kim EK, Noh WC, Han W, Noh DY. Prognostic significance of young age (<35 years) by subtype based on ER, PR, and HER2 status in breast cancer: A nationwide registry-based study. World J Surg. 2011;35(6):1244–53.
18. Trihia H, Murray S, Price K, Gelber RD, Golouh R, Goldhirsch A, Coates AS, Collins J, Castiglione-Gertsch M, Gusterson BA. international breast cancer study group KI-67 expression in breast carcinoma: Its association with grading systems, clinical parameters, and other prognostic factors–a surrogate marker? Cancer. 2003;97(5):1321–1331.
19. Inwald EC, Klinkhammer-Schalke M, Hofstädter F, Zeman F, Koller M, Gerstenhauer M, Ortmann O. Ki-67 is a prognostic parameter in breast cancer patients: Results of a large population-based cohort of a cancer registry. Breast Cancer Res Treat. 2013;139(2):539–552.
20. Petrelli F, Viale G, Cabiddu M, Barni S. Prognostic value of different cut-off levels of Ki-67 in breast cancer: A systematic review and meta-analysis of 64,196 patients. Breast Cancer Res Treat. 2015; 153(3):477–91.
21. Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA. Ki67 in breast

cancer: Prognostic and predictive potential. Lancet Oncol. 2010;11(2):174-83.

22. Bouzubar N, Walker KJ, Griffiths K, Ellis IO, Elston CW and Robertson JF. Ki67 immunostaining in primary breast cancer: pathological and clinical associations. Br J Cancer. 1989;59(6):943–7.
23. Molino A, Micciolo R, Turazza M, Bonetti F, Piubello Q and Bonetti A. Ki-67 immunostaining in 322 primary breast cancers: Associations with clinical and pathological variables and prognosis. Int J Cancer. 1997;74(4):433–7.
24. Kuru B, Camlibel M, Gulcelik MA, Alagol H. Prognostic factors affecting survival and disease-free survival in lymph nodenegative breast carcinomas. J Surg Oncol. 2003;83(3):167–72.
25. Luporsi E, André F, Spyratos F. Ki-67: level of evidence and methodological considerations for its role in the clinical management of breast cancer: Analytical and critical review. Breast Cancer Res Treat. 2012;132:895.
26. Denkert C, Von Minckwitz G. Reply to Ki-67 in breast cancer: A useful prognostic marker!. Array. 2014;25:542-543.
27. Tan QX, Qin QH, Yang WP, Mo QG, Wei CY. Prognostic value of Ki-67 expression in HR-negative breast cancer before and after neoadjuvant chemotherapy. International Journal of Clinical and Experimental Pathology. 2014;7:6862-6870.
28. de Azambuja E, Cardoso F, de Castro G, et al. Ki-67 as prognostic marker in early breast cancer: A meta-analysis of published studies involving 12,155 patients. Br J Cancer. 2007;96:1504.
29. Zhu X, Chen L, Huang B, Wang Y, Ji L, Wu J, Di G, Liu G, Yu K, Shao Z, Wang Z: The prognostic and predictive potential of Ki-67 in triple-negative breast cancer. Scientific Reports. 2020;10(225).

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/58737