

ASSESSING THE ASSOCIATION BETWEEN THE PROLIFERATIVE ACTIVITY OF BREAST- TUMORS EVALUATED WITH KI-67 SCORES AND THE RELATIONSHIP BETWEEN KI-67 SCORES AND MALIGNANT POTENTIAL OF THE BREAST TUMORS

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Type of Study: Original Research Paper

Conflicts of Interest: Nil

ABSTRACT

Background: A non-histone nuclear protein tightly associated with the cell cycle is Ki-67 which is seen in cell cycles phase mid-G1, G2, mitosis, and S phase. However, Ki-67 is not seen in early G1, G0, and resting or quiescent cells. Ki-67 is a reliable indicator of prognosis and proliferation of the tumor in subjects with carcinoma breast.

Aims: The present clinical study was conducted to assess the association between the proliferative activity of breast- tumors evaluated with Ki-67 scores and the relationship between Ki-67 scores and malignant potential of the breast tumors

Methods: The present prospective clinical study included 374 female subjects of the FNAC from subjects having palpable breast lesions. The second pass was given, whereas, the first pass was used for either LBC or conventional smear. Several criteria were used to compare representative LBC and conventional smear. Individual scoring of each criterion was done followed by statistical evaluation. Ki-67 proliferation index was assessed for conventional smears and LBC.

Results: For conventional smear diagnosis, benign cases reported were 253 were negative for Ki-67 immunostaining. Among uncertain cases, 4 cases were negative for Ki-67 immunostaining were 1 case had a low Ki-67 proliferative index and 14 had a high Ki-67 proliferative index. On conventional smear, 74 cases were seen as malignant whereas all the cases had a high Ki-67 proliferative index. In the Ki-67 proliferation index and diagnosis on LBC (liquid-based cytology), it was seen that there were 253 cases reported as benign on LBC which were negative. In the present study, 19 subjects were reported as uncertain,

among which 4 cases were negative for Ki-67 immunostaining, 1 subject had low Ki-67 proliferative index, and 14 specimens had high Ki-67 proliferative index. For the distribution of cases based on Ki-67 proliferation index in the study subjects, the number of cases reported with a score 0 i.e., negative for Ki-67 was 257, number of cases reported with a score of 1 i.e., the low proliferative score for Ki-67 were 01, and number of cases reported with a score 2 i.e., the high proliferative index for Ki-67 were 88

Conclusion: Within its limitations, the present study concludes that the Ki-67 proliferative index is a reliable indicator of prognosis and proliferation of the tumor in subjects with carcinoma breasts.

Keywords: Breast cancer, conventional smear, Ki-67, malignant potential, liquid-based cytology

INTRODUCTION

The incidence of breast cancer is increasing globally including India with a high incidence of 39 cases among every 1,00,000 females with a varying incidence at different geographic regions. The incidence in India is 30 in every 1 lakh subjects and also has a high lifetime risk. Owing to their high burden on the healthcare sector, especially in rural areas, most females with palpable breast lesions don't have access to mammographic investigations. FNAC (fine-needle aspiration cytology) is a widely accepted diagnostic tool used in the majority of cases as it is easy, cheap, requires less equipment, and can be done at almost all the centers. However, another emerging modality is LBC (liquid-based cytology) which is superior concerning even cellular distribution and rapid fixation in a small area on the slide. Hence, the time needed for assessing using cytopathologists and screeners is reduced. Also, the material that remained in fixative liquid can be utilized for immunocytochemistry ancillary studies. The diagnostic efficacy of LBC needs to be explored for fine-needle aspiration biopsy (FNAB).¹

LBC is been used now for a longer time in the field of exfoliative cytology, and for the past few years, LBC is used in FNAB specimens and general cytology studies. Previous literature data initially reported that LBC is suboptimal in the specimens of breast lesions, however, recent studies showed high specificity and sensitivity of LBC to more than 90% in assessing breast lesions. Also, literature data shows a high correlation between conventional smears and LBC samples. LBC allows assessment of nucleolar prominence, smaller cell size, fragmented cell clusters, lost, reduced, or altered background material. LBC uses cell rinsing in a liquid media preservative where sample processing is done on an automated device despite making a smear allowing the cell suspension in a monolayer.²

Similar results were seen in LBC and conventional smears were seen in thyroid lesions and breast lesions. Slight variation in cell morphology can be seen owing to different fixatives. LBC is comparatively expensive. Recently, less expensive methods of LBC have been introduced that can be used in limited-resource settings. However, studies assessing these are limited in the literature. Also, these newer methods do not require refrigeration, and hence, can be used in rural clinics with storage capacity where aspirates are usually fixed poorly and

are degenerated. These shortcomings can be compensated with LBC where the slide is not needed.³

A non-histone nuclear protein tightly associated with the cell cycle is Ki-67 which is seen in cell cycles phase mid-G1, G2, mitosis, and S phase. However, Ki-67 is not seen in early G1, G0, and resting or quiescent cells. Ki-67 is a reliable indicator of prognosis and proliferation of the tumor in subjects with carcinoma breast.⁴

The present clinical study was conducted to assess the association between the proliferative activity of breast- tumors evaluated with Ki-67 scores and the relationship between Ki-67 scores and malignant potential of the breast tumors

MATERIAL AND METHODS

The present clinical study was conducted to assess the efficacy of LBC preparations in breast aspirates for providing well-fixed and adequate diagnostic material and if immunocytochemistry can be applied to cells fixed in this medium successfully. The study population was comprised of the subjects visiting the Outpatient department of the Institute with the complaint of breast lesions. The inclusion criteria for the study were females of age 16 years or more with palpable breast lesions. The exclusion criteria were subjects who were not willing to participate in the study. After explaining the detailed study design, informed consent was taken from all the study subjects in both written and verbal form.

Fine-needle aspiration was done for the breast lump with a 22-gauge needle. For each subject, two passes were done where the first pass was used for the smear preparation conventionally. Under toluidine preparation, adequacy was evaluated on the site for one air-dried smear. 95% ethanol fixation was done for one wet smear immediately for 30 minutes at least, followed by staining with Papanicolaou (Pap) stain. The remaining slides were stained using May-Grunwald-Giemsa (MGG) stain. Both needles were rinsed in a similar LBC liquid medium vial. To assess the adequacy of the FNAB sample, a slide performed in FNAB was stained.

Two different pathologists expertized in their field independently evaluated the FNAB and LBC slides unaware of the results. The samples and specimens were assessed for representative diagnostic material, obscuring factors, and cellularity. Ki-67 proliferation index was assessed for the study subjects. Ki-67 proliferation index and the diagnosis were assessed on conventional staining and liquid-based cytology was done. For assessing the staining, the cell was taken as positive in clearly detectable nuclear staining, whereas, cytoplasmic staining was taken as nonspecific and negative. The scoring of Ki-67 was defined as the percentage of tumor cells that were stained positive from the total malignant cells seen. 10% was taken as the cut-off values to distinguish the high and low proliferative index where $\leq 10\%$ was the low proliferation index and $\geq 10\%$ was the high proliferation index.

The collected data were subjected to the statistical evaluation using SPSS software version 21 (Chicago, IL, USA) and one-way ANOVA and t-test for results formulation. The data were expressed in percentage and number, and mean and standard deviation. The level of significance was kept at $p < 0.05$.

RESULTS

The present clinical study was conducted to assess the efficacy of LBC preparations in breast aspirates for providing well-fixed and adequate diagnostic material and if immunocytochemistry can be applied to cells fixed in this medium successfully. Ki-67 proliferation index was assessed for the study subjects. Ki-67 proliferation index and the diagnosis were assessed on conventional staining and liquid-based cytology was done. On assessing the Ki-67 proliferation Index and diagnosis on conventional smear in the study subjects with breast lesion including usual ductal hyperplasia (UDH), atypical ductal hyperplasia (ADH), and ductal carcinoma in situ (DCIS). It was seen that for conventional smear diagnosis, benign cases reported were 253 was were negative for Ki-67 immunostaining. Among uncertain cases, 4 cases were negative for Ki-67 immunostaining were 1 case had a low Ki-67 proliferative index and 14 had a high Ki-67 proliferative index. On conventional smear, 74 cases were seen as malignant whereas all the cases had a high Ki-67 proliferative index as shown in Table 1.

Concerning the assessment of the Ki-67 proliferation index and diagnosis on LBC (liquid-based cytology), it was seen that there were 253 cases reported as benign on LBC which were negative. In the present study, 19 subjects were reported as uncertain, among which 4 cases were negative for Ki-67 immunostaining, 1 subject had low Ki-67 proliferative index, and 14 specimens had high Ki-67 proliferative index as shown in Table 2.

For the distribution of cases based on Ki-67 proliferation index in the study subjects, the number of cases reported with a score 0 i.e., negative for Ki-67 was 257, the number of cases reported with a score 1 i.e., the low proliferative score for Ki-67 was 01, and the number of cases reported with a score 2 i.e., the high proliferative index for Ki-67 was 88 as depicted in Table 3.

DISCUSSION

The present clinical study was conducted to assess the efficacy of LBC preparations in breast aspirates for providing well-fixed and adequate diagnostic material and if immunocytochemistry can be applied to cells fixed in this medium successfully. Ki-67 proliferation index was assessed for the study subjects. Ki-67 proliferation index and the diagnosis were assessed on conventional staining and liquid-based cytology was done. On assessing the Ki-67 proliferation Index and diagnosis on conventional smear in the study subjects with breast lesion including usual ductal hyperplasia (UDH), atypical ductal hyperplasia (ADH), and ductal carcinoma in situ (DCIS). It was seen that for conventional smear diagnosis, benign cases reported were 253 was were negative for Ki-67 immunostaining. Among uncertain cases, 4 cases were negative for Ki-67 immunostaining were 1 case had a low Ki-67 proliferative index and 14 had a high Ki-67 proliferative index. On conventional smear, 74 cases were seen as malignant whereas all the cases had a high Ki-67 proliferative index. These results were consistent with the studies of Pinto AE et al⁵ in 2001 and Buhmeida EEA et al⁶ in 2012 wherein their studies authors showed similar Ki-67 proliferation Index and diagnosis on conventional smear as seen in the present study.

For the assessment of the Ki-67 proliferation index and diagnosis on LBC (liquid-based cytology), it was seen that there were 253 cases reported as benign on LBC which were

negative. In the present study, 19 subjects were reported as uncertain, among which 4 cases were negative for Ki-67 immunostaining, 1 subject had low Ki-67 proliferative index, and 14 specimens had high Ki-67 proliferative index. These results were in agreement with the studies of Trihia H et al⁷ in 2003 and Urruticoechea A et al⁸ in 2005 where authors reported comparable results for the assessment of the Ki-67 proliferation index and diagnosis on LBC as in the present study.

Concerning the distribution of cases based on Ki-67 proliferation index in the study subjects, the number of cases reported with a score 0 i.e., negative for Ki-67 was 257, the number of cases reported with a score 1 i.e., the low proliferative score for Ki-67 was 01, and the number of cases reported with a score 2 i.e., the high proliferative index for Ki-67 was 88. These results were comparable to the studies of Kaem B et al⁹ in 2011 and Ferguson NL et al¹⁰ in 2013 where authors reported comparable distribution of subjects in their studies based on the proliferation of the Ki-67 index.

CONCLUSION

Within its limitations, the present study concludes that the Ki-67 proliferative index is a reliable indicator of prognosis and proliferation of the tumor in subjects with carcinoma breast. However, the present study had a few limitations including a small sample size, shorter monitoring period, and geographical area biases. Hence, more longitudinal studies with larger sample size and longer monitoring period will help reach a definitive conclusion.

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TABLES

Conventional smear diagnosis	Li-67 proliferation Index		
	00 Negative	01 Low ($\leq 10\%$)	02 High ($\geq 10\%$)
Benign	253	00	00
Uncertain	04	01	14
Malignant	00	00	74
Total	257	01	88

Table 1: Ki-67 proliferation Index and diagnosis on conventional smear in the study subjects with breast lesion

Conventional smear diagnosis	Ki-67 proliferation Index		
	00 Negative	01 Low ($\leq 10\%$)	02 High ($\geq 10\%$)
Benign	253	00	00
Uncertain	04	01	14
Malignant	00	00	74
Total	257	01	88

Table 1: Ki-67 proliferation Index and diagnosis on LBC in the study subjects with breast lesion

Ki-67 proliferation Index	Score	Percentage (%)	Number (n)
Negative	0	74.3	257
Low ($\leq 10\%$)	1	0.3	01
High ($\geq 10\%$)	2	25.4	88

Table 3: Distribution of cases based on Ki-67 proliferation index in the study subjects