Original Article

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Effectiveness of Special Training on Achieving Reproducible Mitotic Counts in Malignant Tumors

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ABSTRACT

Background: Mitotic count is the most commonly used method of assessing the proliferative activity of a tumor. It is used for classification, grading, prognostication of tumors and sometimes as a decision factor for treatment. Many variables related to procedure or person can influence the mitotic count. This study is designed to find out the effectiveness of special training in mitotic counting in achieving reproducible results.

Methods: Sections from twenty cases of carcinoma breast formed the study material. Five junior residents were given the same twenty sections counted by the principal investigator for mitosis counting and original counts were recorded. Then they were instructed regarding mitotic figures using written material and graphic representations. All of them were asked to count the mitotic figures in the same section once again. The results of the two sets were compared. Paired t test and linear regression were the statistical tests used in analysis. Data storage and Analysis is done with EPIINFO software.

Results: The mean standard mitotic counts obtained by principal investigator were 8.21. The mean original mitotic count obtained by residents was 15.79. After attending the training program the mean mitotic counts obtained by residents dramatically came close to that obtained by principal investigator (8.43)

Conclusion: In trained hands, the mitotic counts are fairly reproducible and give results comparable to more sophisticated methods of determining proliferative activity in breast cancer.

Keywords: *Mitosis, Training, Residents*

Introduction

Worldwide breast cancer remains the most common cancer diagnosed in women Approximately 1.8 million new cases are diagnosed per year and incidence is rising by every year.[1] After the introduction of mammographic screening large numbers of early stage cancers have been detected and therapeutic options for primary breast cancer has changed significantly. It is the duty of the pathologist to provide the clinicians accurate prognostic information for selection of the optimum therapy for each patient. Histological grade assessed by Nottingham system / modified Bloom–Richardson–Elston grading system determines prognosis in women with operable breast carcinomas. Mitotic counts, which assess the proliferative activity of the neoplastic tissue is one of the three factors measured by this grading system

Mitotic counting is the old and classic method used to assess the proliferative activity of normal and neoplastic tissues. It is done by counting mitotic figures in H& E sections. Even though simple, this method may not be accurate always. The number of mitotic figures depends upon the time passed between surgical removal of the specimen and formalin fixation.[2-3] Fixation is the first step in tissue preparation for microscopic analysis. A well fixed tissue is a pre requisite for a good slide and so for a good diagnostic interpretation. Our previous study already showed that lack of prompt fixation led to significant change in mitotic counts. Both mitotic counts and ki-67 index were significantly higher in immediately fixed specimens compared to those fixed after 1 hour. [4] Another problem is mitotic figures which are visible in histological sections indicate only the M phase of the cell cycle, because of this the cells in other phases of mitosis can be easily missed.

Routinely mitotic counts are reported as number / 10HPF. But because of variation in the size of high power field mitotic counts may vary up to 250%. [5] Another problem can occur due to tumor heterogeneity leading to non uniform distribution of mitotic figures. Malignant tumors being heterogeneous, different part of tumors will show different proliferative activity highest seen in the invading front. So the mitotic counts can vary in different slides taken from the same tumor also.[6-7] And again when counting the mitotic figures, apoptotic cells and neutrophilic granulocytes can mimic mitotic figures. So it is also necessary to adhere to strict morphological criteria.[8-10] Mitotic counts will also vary according to section thickness. All these factors can

result in false counts and also in poor reproducibility of mitotic counts leading to false prognostic information about the patient.

Even if all the known above mentioned procedural factors are standardized error can also occur at the individual level who interpret the counts. Two types of observer variation are inter-observer variation and intra-observer variation. In an well fixed adequately sampled tumor sections the most perplexing question will be what should be called as a mitotic figure. Many dark irregular structures will be there in the sections which will be confused with mitotic figures. It is known that only definite mitotic figures should be counted and apoptotic cells and degenerate nuclei should be excluded. [11] Similar to any other technique special training is needed in this scenario also and it will be more helpful in making mitotic counts more reproducible.

In one previous study when same stained slide of a smooth muscle tumor was circulated among experienced pathologists results obtained were dramatically inconsistent. The counts varied from 0 to 22.[12] In another study where both pathologists and specially trained technicians participated in evaluating the variation in mitotic counts in sections of breast cancer. The participants counted the mitotic figures in twenty samples in ten high power fields. Experienced pathologists showed the highest variation in mitotic counts and specially trained technicians had lowest variation. In groups without special training for mitotic counts, the mean grading efficiency was low. Experienced pathologists had the potential to grade 88% of all the cases correctly where the trained technicians correctly graded 95% of the cases.[7]

In our present study we tried to find out the how the training and tutoring regarding criteria for mitosis affected the reproducibility of counts by standardizing all other interfering procedural factors like fixation time, section thickness, microscope used etc

Materials and method

Study conducted in the Department of Pathology, Sree Narayana Institute of Medical Sciences during 2015. Our study sample included 20 cases of Infiltrative duct carcinoma. The specimens were collected from the operation theatre immediately after removal. They were cut and examined and specimen immersed in fixative after 1 hour and sent for routine grossing. Multiple sections at 4-5 micrometer were taken in all cases for comparison. Mitotic counts were made by principal investigator in Labomed microscope with high power field having 0.1325 mm^2 area. Counting is done in a systematic fashion starting from a field in which the first mitosis is seen on eyeballing and counted 20 consequent high power fields. A typical basophilic metaphase in a clear slightly basophilic / eosinophilic background can be regarded as a mitotic figure. The counts made by the principal investigator on 4-5 micrometer thick sections on a Labomed microscope using systematic counting method in sections fixed 1 hour after removal will be considered as the standard.

To study the effect of tutoring / training regarding criteria for mitosis on the reproducibility of mitotic counts, five junior residents were given the same 20 sections counted by the principal investigator for mitosis counting and original counts were recorded. Then they were instructed regarding mitotic figures and their distinction from apoptosis and degenerate nuclei, using written material and graphic representations. They were instructed to start We followed the strict criteria for identifying mitotic figures proposed by Multicentre Morphometric Mammary Carcinoma project. According to this criteria in a mitotic figure, the nuclear membrane should be absent and clear hairy extensions of nuclear material should be present, either clotted, in a plane, or in separate clots. Two parallel clearly separated chromosome clots can also be taken as mitotic figures. Also all mitotic mimickers like apoptotic bodies, pyknotic nucleus, lymphocytes, mast cells and degenerate cells should be excluded (figure 1,2).All of them were asked to count the mitotic figures in the same section once again after the training section.. The results of the two sets (Pre and post instruction) were compared. Paired t test and linear regression were the statistical tests used in analysis. Data storage and analysis is done with EPIINFO software

Results

Sections from twenty cases of carcinoma of breast formed the study material. Most of the cases of carcinoma breast (85%) were grade 2 tumors. There was one (5%) grade 1 and two (10%) grade 3 tumors .When sections from twenty cases of carcinoma of breast were analyzed for mitotic counts, the mean counts obtained by principal observer was 8.21. Then five junior residents were selected and given the same twenty sections for mitotic counting and first set of counts were noted. The mean original mitotic count obtained by residents before instruction was 15.79. The values varied from 13. 37 to 18. 22. All of them were asked to recount the mitotic figures in the same section once again after attending a session about mitotic counting. After this training program the mean count obtained dramatically came very close to that obtained by principal investigator (8.43) and range also narrowed (7.14-9.76). Results are summarized in table 1

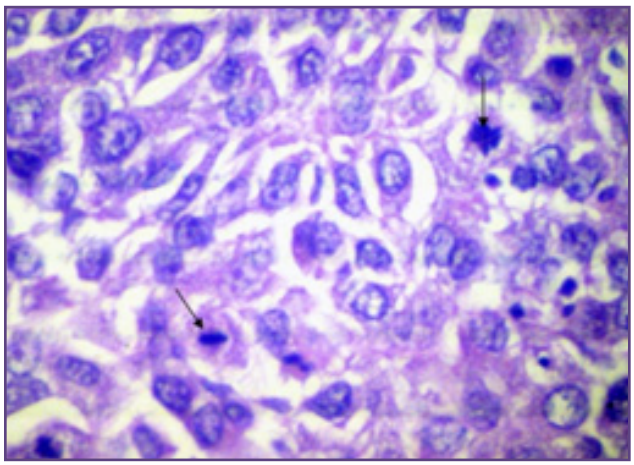

Fig. 1: Mitotic figures in a high grade breast carcinoma. Note the clear or lightly stained cytoplasm of mitotic cells. H&E x 400.

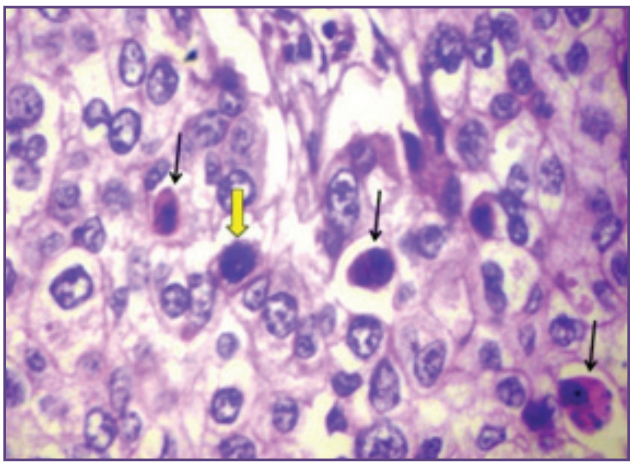

Fig. 2: Apoptotic bodies [black arrow] have intensely eosinophilic cytoplasm and retraction artifacts around them. Mitotic figures [yellow arrows] have to be distinguished from apoptotic bodies [black arrows]. H&E x 400.

Table 1: Comparison between mitotic counts obtained before and after instruction.

Parameter	Mean (95% CI)	Paired t test	
		t	p
Residents before instruction	15.79 (13.37 - 18.22)	6.90	<0.001
Residents after instruction	8.45 (7.14-9.76)		

Discussion

Mitotic count plays an important role in diagnosis and prognosis of malignant tumors**.** Mitotic counts forms one of the factors assessed in grading of carcinoma of breast by modified Bloom Richardson grading system. Proliferation index is also forms most important prognostic indicator in melanoma, sarcoma as well as in hematopoietic malignancies. Counting mitosis and expressing it as a quantitative figure per a set number of high power fields is a time honored method used by histopathologists in the assessment of cell proliferation. Many of the factors associated with method, instrument and observer can result in poor reproducibility of this technique. Even though all factors are standardized, mitotic counting is considered to be tedious, time consuming and thoroughly boring procedure and a technique requiring intuition, tution and practice. After keeping a strict and standardized protocol for specimen fixation, processing, cutting and staining, significant difference can still occur in mitotic counts due to interobserver variation. In our present study we tried to find out how the special training helps the pathologist to reduce interobserver variation and to achieve reproducible mitotic counts.

The mitotic counts obtained by Principal investigator are taken as standard count (8.21). The original counts done by junior residents were seemed to be almost double of that of the standard count (15.79). It is clear that they were overestimating the counts. The basic problem lies in understanding what actually constitute a mitotic figure. So they were asked to attend a tutoring session about mitotic counting where they were instructed about the criteria regarding a mitotic figure. Appearance of mitotic figures will vary according to the phase in which the dividing cells were present during the time of fixation. According to the standard criteria, there should be hairy extension of nuclear material without a nuclear membrane. Cells in mitotic phase will have abundant cytoplasm. We should be able to see at least one chromosomal end. Always count two clearly separate parallel chromosome clumps as single mitotic figure, not as two. (Figure 1)

Another problems lies in differentiating mitotic figures from mimickers of mitotic figures like apoptotic bodies, degenerate pyknotic cells, neutrophil nuclei and mast cells. Apoptotic cells will appear as shrunken cells with fragmented nuclei showing condensed chromatin .They will have intensely eosinophilic cytoplasm and seen to be surrounded by retraction artifacts. Inflammatory reaction will be characteristically absent around them. (Figure 2) Next doubt was which area of the section should be selected for mitotic counting. Malignant tumors being heterogeneous, mitotic activity will also be different in

different areas of the tumor. It is seen that invading front of the tumor show maximum proliferative activity. So in sections taken from these areas counting should start from an area where the mitotic figures are first seen on eyeballing.

When the junior residents recounted the mitotic figures after attending the session, mean counts came down very to close to standard counts (8.43). This shows the effectiveness of prior instruction in improving the precision of mitotic counts. (Table 1) The correlation is also found to be very poor for the observer variability between the investigator and residents. However it is to be remembered that it were the junior residents who were used in the experiment. The results would probably have been less dramatic with more experienced pathologists.

Even though more specific markers of proliferation like MIB labeling or Ki -67 index is available today still it is important for all of us to follow a standardized protocol for mitotic counting. When done on H& E sections with caution and good knowledge, mitotic counts gives a precise idea about proliferative activity since cost of newer technique are still unaffordable to vast majority of our patients .

Conclusion

Mitotic count indicating the proliferative activity of a tumor is subjected to errors related to the procedural and observer factors. In a well fixed adequately sampled tumor sections the apoptotic cells and degenerate nuclei can mimic mitotic figure, and a strict morphological criteria is essential to reach reproducible results. Similar to any other technique special training is helpful in making mitotic counts more reproducible. In trained hands, the mitotic counts are fairly reproducible and give results comparable to more sophisticated methods of determining proliferative activity in breast cancer.

Acknowledgement

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Ethical Approval

Informed consent already taken during the surgery

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