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Original Research Article

A comparison of Ki67 index in carcinoma breast by digital image analysis with manual method

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ABSTRACT

Introduction: Ki67 labeling index (LI) for breast carcinoma is essential for the therapy. It is done by visual assessment under the microscope which is subjective, hence has limitations of inter-observer variability. A standardized method for evaluating Ki67 LI is necessary due to reduce subjectivity and improve precision. Hence automated Digital Image Analysis (DIA) has been attempted as one of the potential methods for evaluating Ki67 index.

Materials and Methods: 48 cases of invasive breast carcinoma were included in this study. Ki67 immunostaining was carried out on paraffin embedded sections of all cases. Visual assessment (VA) of Ki67 index was carried out and images of Ki67 stained slides were obtained under a magnification of 40X. The mean value of two observers was considered as final visual assessment scores. The images were processed using Tissue Quant software to pick up positively stained areas. The area and count of the positively stained regions were obtained. Statistical analysis by calculating kappa value was performed to check the agreement between DIA and visual assessment.

Results: Out of 48 cases, 40 cases showed substantial agreement between DIA and visual analysis established by calculating kappa value (0.636).

Conclusion: DIA is an emerging and competitive alternative for biomarker evaluation in breast cancer and can be effectively put in use for clinical practice to obtain precise results.

1. Introduction

Breast cancer, among women, continues to be the most common cause of death due to cancer worldwide.[1] Hence assessment of prognosis is an important aspect in clinical practice. The hallmark of all tumors is uninhibited abnormal cell proliferation. Ki67, a nuclear antigen, is a cell proliferation marker expressed in all but G_0 phase of cell cycle.[2] With the advent of molecular profiling, ki67 LI has emerged as a potential prognostic marker in breast cancer.[3] Many of the studies have validated the importance of ki67 LI correlation with breast cancer outcome particularly in patients after hormonal and chemotherapy.[2] Also it has been suggested that ki67 LI can aid in identification of patients who could be benefited from chemotherapy.[4] Currently majority of the institutions and laboratories follow visual assessment method to quantify ki67 index owing to the fact that it is economical. However, visual assessment is tedious and poses challenges like intra and inter-observer variability. Furthermore, no standardized scoring system has been established so far to designate a tumor as highly proliferative or not. Hence, a standardized method is necessary to evaluate ki67 index due to its impact on clinical practice. Digital image analysis is emerging as a potential alternative to visual assessment as it is less time consuming, lacks manual error and is capable of processing the images more accurately.[5,6] Various recent studies have shown a high concordance rate between visual assessment and digital

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image analysis of ki67 LI. The present study was conducted to compare the visual assessment results of ki67 LI with Tissue Quant image analysis software.

2. Materials and Methods

The present study was a two year study from June 2016 to May 2018 conducted in a tertiary care hospital. A total of 48 cases diagnosed as invasive breast carcinoma on histopathology were included in the study. Formalin fixed paraffin embedded tissues were used for ki67 LI in all the cases.

2.1. Immunohistochemistry

Formalin fixed paraffin embedded sections of 3μm thickness were cut for all 48 cases and processed for immunohistochemistry. Ki67 immunostaining was done using mouse monoclonal primary antibody MIB-1 with a dilution of 1:100 and a secondary antibody kit PolyExcel HRP/DAB Detection system by PathnSitu Biotechnologies. Visual assessment of ki67 LI was done by counting a total of 500 cells under 40X magnification and calculating the percentage of positively stained cells. Two observers independently calculated the values for all cases and an average of both values was taken as final ki67 score by manual method.

For Digital image analysis, images of the same areas screen ed for manual method were taken. Images were acquired at a magnification of 40X using Lawrence brand of microscope. The resolution of the images was 4608 x 3456 pixels. These images were subsequently processed for automated analysis using software named TissueQuant which picks the regions of a specific color of interest in an accurate manner. The brown shade corresponding to the positively stained nucleus was considered as the reference color while performing the analysis. TissueQuant software provides a batch process option using which all the input images were analyzed. From each image, the count of the nuclei and the total area represented by all the positively stained nuclei were obtained through this analysis. As a second step, the area representing the field of view in each image was also obtained by using the background pixel color as the reference color for TissueQuant analysis. The ratio of the total area of the positively stained nuclei to the area of the field of view was considered as a measure to be correlated with the manual scoring.

Statistical analysis for the data was done by calculating kappa value to analyze agreement between manual method and DIA. The cut off value for categorizing the cases into low and high proliferation rate was 14.

3. Results

In the present study, 48 cases were evaluated by visual assessment of ki67 by two observers as well as by DIA using TissueQuant software. The DIA calculated the percentage area of positivity in the given image. Out of 48 cases assessed, 40 cases showed considerable concordance which was established by calculating the kappa value. The image taken manually (1) and digital image of the same (2) are shown below graph was plotted with two readings of manual, average of the two and DIA values as depicted below(3).

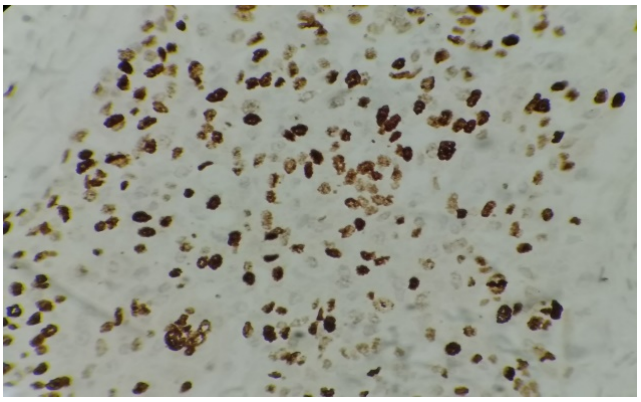


Fig. 1: Ki67 immunostaining image under 40X magnification

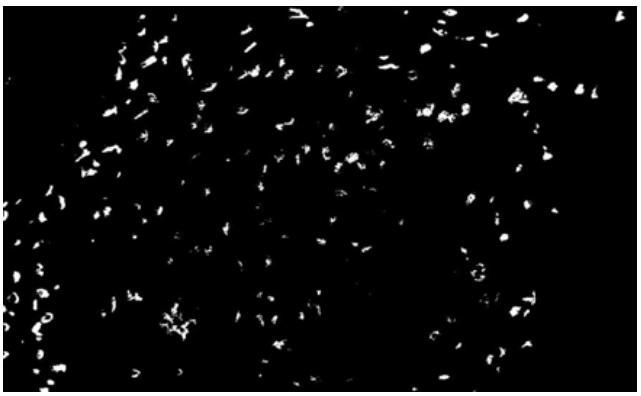
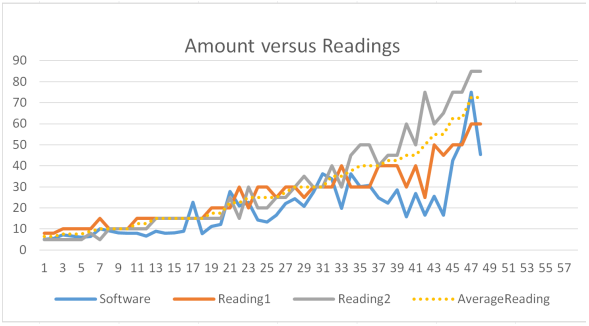


Fig. 2: Matching with digital image analysis of the same image

Inter observer variability between two readings R1 and R2 was evaluated with P value of 0.862. Kappa value was calculated between averages of these two readings and DIA readings. A value of 0.636 was obtained, which shows there is substantial agreement between the two methods. Out of 48 cases, 8 cases showed discordance between DIA and manual values. These cases showed values more than 14 by manual method and less than 14 by DIA. This discordance is likely to be due to smaller area in the image.

4. Discussion

The present study was carried out to evaluate the agreement between ki67 values by visual assessment and DIA. Since ki67 is an important prognostic marker in breast cancer, the



Graph 1:

values obtained were categorized into two groups' i.e. high proliferation and low proliferation. Presently, ki67 index above 14 is considered high proliferation for therapeutic purposes, hence cut off value for this categorization was taken as 14. Values above 14 were taken as tumors having high ki67 index and below 14 were taken as tumors having low ki67 index. In our study we found that out of 48 cases, 40 cases showed agreement between manual method and DIA values. In 8 cases the DIA values were lower as compared with manual values. This discrepancy could be due to variation in the image sizes which in turn leads to lower area/pixel calculation, hence lower values were obtained by DIA. Faded slides and those with excessive background staining were excluded from the present study.

A similar study done by Tuominen et al using Immunoratio software showed good correlation between manual and DIA values.[7] Another study done by Stalhammer et al showed that DIA values were more accurate as compared to manual values.[1] Koopman T et al performed DIA using virtual dual staining which showed excellent inter-platform agreement between two independent DIA platforms.[6] Likewise, present study also showed good agreement between the two methods.

The TissueQuant software used here is a simple, cost effective and easy tool for DIA. In the present study, images were taken using mobile camera with reasonably good resolution to obtain fairly accurate values.[8] Furthermore in institutions where automated DIA machines cannot be put in use due to financial constraints, this software proves to be economical. Visual assessment is subjective and has possibility of higher inter observer variability, though not recorded in our study possibly due to smaller sample size. Imaging technique needs to be uniform and a standardized method can be practiced to avoid any such discrepancies as encountered in 8 out of 48 cases in our study. Also calibration of the software can be done to cater to specific sized images.[9] This method of DIA can further be extrapolated to other cancers to evaluate ki67 index, such as is in oropharyngeal squamous cell carcinoma.[10] Furthermore, this TissueQuant software can also be used to assess other immunohistochemistry markers and quantify them. TissueQuant want used to assess ER, PR and Her2 neu status and grade them with higher accuracy.[8]

5. Conclusion

A highly studied biomarker, ki67, has proven prognostic value in breast cancer. Tumors having high ki67 index have poorer prognosis as compared to low grade tumors with low ki67 index.[11] As it is a marker of proliferation index, stratification of the tumors into one of the two categories is essential. Using TissueQuant software for DIA, not only such stratification is possible but can also yield specific ki67 index. Thus we conclude that DIA is a promising and competitive alternative for biomarker evaluation in breast cancer and can be effectively put in use for clinical practice.

6. Source of funding

None.

7. Conflict of interest

None.

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