Comparison of estrogen receptor, progesterone receptor and Ki-67 immunohistochemistry scoring using manual method and image analysis in breast carcinoma cases

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Abstract

Background: Breast carcinoma is the leading cause of carcinoma death in women. Estrogen receptors(ER), progesterone receptors(PR) and Ki-67 are important prognostic determinators. However immunohistochemistry (IHC) reporting always has a degree of subjectivity and interobserver variability. This study was done to validate digital image analysis of IHC images using Immunoratio web plugin and if it can overcome the above difficulties. **Objectives:** To score ER, PR and Ki 67 immunostaining by manual light microscopy and image analysis software. To compare the scoring done by manual method versus image analysis. **Methods:** 50 breast carcinoma IHC slides each of ER, PR and Ki67 performed at the Department of Pathology, Sri Devaraj URS Medical College from year 2009- 2014 was be taken up for study. IHC was done with Biogenex Antibody using standard IHC protocol. The IHC slides were reported by two pathologists and micrograph of 3 select areas were analysed using Immunoratio image analysis software and the results were compared. Analysis software uses multistep process for quantitative analysis and colour histogram module for intensity analysis. **Results:** IHC image analysis had a sensitivity and specificity of more than 90% in scoring ER and PR as positive and negative using Allred Score. However with Ki 67 the sensitivity was more than 80% only when the cut off was selected as 20%. **Conclusion:** Immunoratio image analysis software can be used to assist the pathologist in scoring IHC slides in reducing the subjective error rather than completely replacing manual scoring of IHC images. It will continue to be a good alternative to whole slide imaging with image analysis software in developing countries.
Key Words: Breast carcinoma, Image analysis, ER,PR, Ki 67, Immunoratio.

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INTRODUCTION

Breast carcinoma is most common malignant tumor and leading cause of carcinoma death in women.[1] Majority of the breast cancers are hormone dependent and hence

Estrogen and Progesterone receptor testing is now a routine protocol for breast carcinoma. Estrogen receptors (ER) and progesterone receptors (PR) in the tumor tissue correlate well with response to hormone therapy and chemotherapy. Hence being strong predictive markers in breast cancer management.[2]Cell proliferation index by Ki-67 has an important prognostic determinator and evident from the fact that mitotic activity is incorporated into the microscopic grading. However many studies done in the past have shown immunohistochemistry (IHC) reporting has a degree of subjectivity and interobserver / interlaboraratory variability. This has resulted in false negative rate which may result in inappropriate management of the patient. Many image analysis softwares are currently available which claim a high degree of accuracy and precision. However most of

these are expensive as they are bundled with particular image acquisition devices or need whole slide imaging which increases the cost and hence not cost effective in most institutes in developing countries. There are currently few free image analysis softwares but their accuracy and precision hasn't been well documented. For this study we chose immune ratio a web application which can be run on any operating system and in most commonly used browsers such as firefox, safari, chrome and internet explorer. This study was done to validate digital image analysis of IHC images using a Immuno ratio a free image analysis software with manual pathologist scoring and if it can give a precise reports.

SAMPLE: Sample size was calculated using nmaster software using correlation co-efficient of study and taking error (5%), power (90%) and standard deviation[2]. A minimum sample size of 47 cases was obtained.[3] Sample size of 50 IHC slides each of ER, PR and Ki 67 was taken.

MATERIAL AND METHODS

50 breast carcinoma IHC slides each of ER, PR and Ki67 performed at the Department of Pathology, Sri Devaraj Urs Medical College from year 2009- 2014 was taken up for study. ER, PR and Ki67 was done using Biogenex Antibody using standard IHC protocol. The IHC slides were reported by two pathologists and micrograph of 3 select representative areas taken at 400x magnification was analyzed using Immunoratio web based plugin and the results compared. ER and PR was scored by Allred Scoring System. Allred scoring was calculated by adding the proportion score and intensity score. A value 0-2 was considered negative and scores between 3-8 was considered positive. Ki67 Quantitative Scoring was done and the percentage of staining cells was obtained. Ki 67 scores below 20% was considered negative and above that was considered positive. The results obtained by manual method was considered as the gold standard and every case was reported by two pathologists individually and a consensus report was obtained. For image analysis microphotograph was captured using Axiocam ERc 5s CMOS sensor mounted on Primostar Zeiss microscope using Zen Blue software at 400x magnification. Images stored in jpeg format and uploaded into the web plugin. The software colour corrects the image using a bank image using colour adjustment wizard which adjusts the contrast and brightness of the image to optimum levels. Immunoratio is an image analysis software built on image j analysis software and uses color deconvolution algorithm, adaptive thresholding and area segmentation for quantitative analysis.[4] Colour histogram module will be used for intensity analysis based on the principle that every colour in an RGB image mode has an unique identity and colour intensity varying from 0 to 255. The colour thresholding and water shed algorithm helps to separate overlapping nuclei for accurate counting.[5-12] The application then displays the percentage of positive cells in the screen as shown in Fig 1,2 and 3

Statistical Analysis: Sensitivity, specificity, positive predictive value and negative predictive value for IHC image analysis was calculated. Agreement between manual and image analysis was obtained by using Cohen's Kappa.
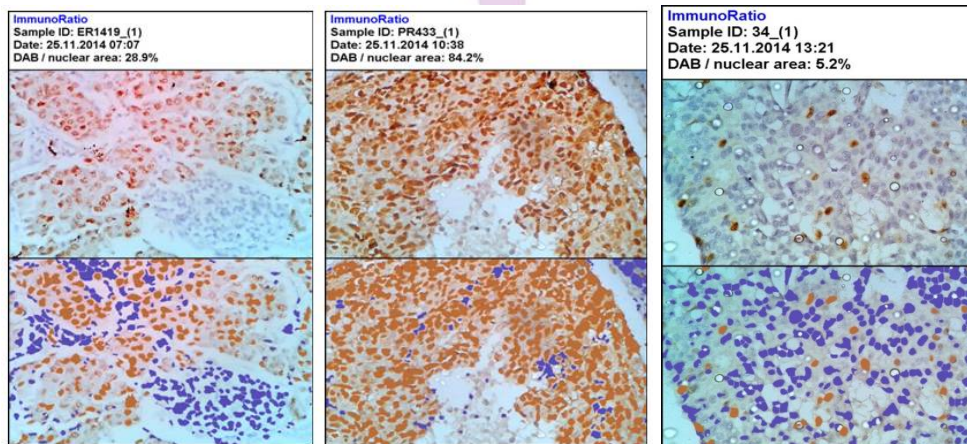
RESULTS

Figure 1:   **Figure 2:**   **Figure 3:**

Figure 1: IHC stained image above and Immuno ratio assessed image below for Estrogen receptor IHC slide scored 3 for 10-33% ER positive tumor cells and similar result on Immunoratio. **Figure 2:** IHC stained image above and Immuno ratio assessed image below for Progesterone receptor IHC slide scored 5 for 66-100% PR positive tumor cells and similar result on Immunoratio. **Figure 3:** IHC stained image above and Immuno ratio assessed image below for Ki 67 receptor IHC slide scored negative with than 20% Ki 67 positive tumor cells and similar result on Immunoratio

All of the breast carcinoma cases included in the study were female with mean age of 46.4 years ranging from 31 to 65 years of age. The interpathologist agreement prior to consensus was 86/90/84 percent for ER/PR/Ki 67 respectively. After verification and consensus by the two pathologists the score was considered to be final score. Immunoratio image analysis software for ER analysis had a sensitivity and specificity of 94 and 100% with PR scores showing 100 % sensitivity and specificity. As expected Ki 67 had a lower sensitivity of 81.4% and specificity of 96% as indicated in

Table 1: Sensitivity, Specificity and cohens kappa value of Image analysis

Image Analysis	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Cohens kappa value	Interpretation (agreement)
ER	94	100	100	96	0.453	Moderate
PR	100	100	100	100	0.652	Substantial
Ki 67	81.4	96	96	96	0.796	Substantial

DISCUSSION

Manual scoring of IHC has been done using various scoring systems. ER and PR are being scored using the Allred scoring system which has a proportion and intensity score. Despite the objective scoring guide many cases present with difficulty in confident, accurate scoring leading to a degree of subjectivity in IHC scoring. Image analysis using immune ratio not only significantly reduced the time taken to analyze the slide but offered an objective scoring which can be recorded for further reference. The cohens kappas value also showed moderate to substantial (good) agreement between manual and automated methods. Many studies have been done comparing the concordance and accuracy of image analysis with manual scoring by pathologist. Similar results have been obtained by other studies with a high agreement rate and kappa value indicating substantial to very good strength of agreement between manual IHC scoring and scoring by image analysis.[13-15]

CONCLUSION

IHC Image analysis is fast and precise most of the times but accuracy of scoring requires proper selection of area, adequate counterstaining, calibration, background correction and many other variables which when not corrected gives inaccurate results. Hence we suggest that image analysis software will assist the pathologist in scoring IHC slides in reducing the subjective error rather than completely replacing manual scoring of IHC images. It will continue to be a good alternative to whole slide imaging with image analysis software in developing countries.

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