Validation of Fine Needle Aspiration Cytology in the Evaluation of Human Epidermal Growth Factor Receptor-2 and Hormonal Receptor Expression Patterns in Breast Cancer Patients

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Abstract
Introduction: Breast cancer is defined as a group of malignant neoplasms and accounts for up to 22% of all female cancers. It rarely occurs in men. In breast cancer, the level of human epidermal growth factor receptor-2 (HER2) overexpression is said to be a prognostic molecular marker that is used for selection of patients for targeted HER2-therapy. Estrogen receptor (ER) and progesterone receptor (PR) are both prognostic and predictive markers for response to hormonal therapy. Fine-needle aspiration (FNA) provides highly cellular sample. Assessment of HER2, ER and PR status in FNA samples is very important clinically. The study was aimed to validate that fine needle aspiration cytology can be used to assess HER2, ER and PR expression patterns in patients with breast cancer.

Methods: Cell blocks were prepared from FNA material collected from 39 newly diagnosed breast cancer patients and immunocytochemistry (ICC) for HER2 and the hormonal receptors, ER and PR was done. Immunohistochemistry (IHC) for HER2, ER and PR was also done on the corresponding biopsy sections. Both positive and negative quality controls were included in the experiments. The Allred scoring system was used to determine the positivity for PR and ER. The overexpression of HER2 was assessed using a scale of 0-3+ for both proportion and intensity whereby 3+ and above was considered positive. The cell block results were compared with core biopsy results and breast cancer classified into various types as luminal A, luminal B, HER2 over expression and the triple negative. The results were then compared with those of core biopsy immunohistochemistry using ANOVA. Kappa statistics was done to check the level of agreement.

Results: Cell block and biopsy results were compared, for ER there was a concordance of 32/35(91.4%) r=0.842 Sensitivity of 83.3% and specificity of 85.0%. For PR the concordance was 32/35(91.4%) r=0.842 with sensitivity of 84.2% and specificity of 84.2%. For HER2 the concordance was 34/35 (97.1%) r=0.925 with a sensitivity of 88.9% and a specificity of 96.3%. There was moderate agreement between the two methods, k=0.719, p<0.001

Conclusion: The results obtained from FNA cell blocks are reliable when compared with pairedparaffin embedded tissue blocks. Therefore, HER2, ER and PR can be adequately assessed using cell blocks prepared from FNA material.

Keywords: Fine needle aspiration cytology (FNA), Immunocytochemistry (ICC), Immunohistochemistry (IHC), human epidermal growth factor receptor 2 (HER2), estrogen receptor (ER), progesterone receptor (PR), breast cancer
Introduction
Breast cancer is a malignant tumor of the breast and comprises of a diverse group of neoplasms accounting for about 22% of all female cancers. Over 1 million new cases are diagnosed annually resulting to about 400,000 breast cancer related deaths. It is the most prevalent cancer among women. It rarely occurs in men accounting for approximately 1%. The overexpression of HER2/neu in breast cancer is a prognostic marker used to select patients for HER2 therapeutics and has been reported in approximately 20–30% of breast cancer. The up regulation of HER2 is associated with poor prognosis as well as an aggressive disease course. The steroid hormonal receptors for estrogen receptor (ER) and progesterone receptor (PR) are both prognostic and predictive markers for response to hormonal therapies. The clinical-pathological parameters have been used for a long time to determine therapeutic management of patients with breast cancer. Studies indicate that breast cancer HER-2, ER and PR positive are difficult to treat and it is therefore believed that careful patients’ stratification using these biomarkers would improve therapeutic outcomes. Tissue biopsy blocks have been the sample of choice for the assessment of HER2, ER and PR status. However, the cost of processing biopsies is very high especially in developing countries. Sometimes it might be impractical to obtain a surgical excision for HER2, ER and PR testing especially, in patients receiving neoadjuvant therapy or those with inoperable metastases. In such cases, it will be wise to assess HER2, ER and PR status using fine-needle aspiration cytology (FNAC).

Breast cancer is diagnosed by the triple assessment (clinical assessment, imaging and FNA and biopsy). Breast cancers are classified into different subtypes based on their HER2 and hormonal receptor status as obtained by immunohistochemistry results as luminal A (ER+,PR+, HER2-), luminal B (ER+,PR+, HER2+), HER2 over-expression (ER-, PR-, HER2+) and the triple negative type. Studies performed in Kenya have often utilized formalin fixed paraffin embedded tissues (FFPE) despite their limitations in preoperative treatment. The assessment of HER2, ER and PR expression patterns using FNAC has not been adequately explored especially the stratification of breast cancer subtypes based on ER/PR and HER2 expression patterns. In developing countries where resources are limited, determination of these bio-markers on FNAC samples of breast cancer is of clinical significance. FNAC being minimally invasive has been proven to be safe, cheap and fast as compared to core needle biopsies (CNB) and can quickly characterize ER, PR and HER-2/neu status hence providing both diagnostic and prognostic applications. FNAC is accepted worldwide for the screening and diagnosis of breast cancer. Studies show that the evaluation of ER and PR status by immunocytochemistry on cell blocks obtained by FNAC highly correlates with results from tissue blocks as well as biochemistry results. The purpose of this study was to determine the utility of fine needle aspiration cytology (FNAC) in the evaluation of HER2, ER and PR expression patterns compared to core needle biopsies (CNB) in breast cancer patients.

Methods
This was a prospective study including 39 newly diagnosed breast cancer patients referred to Kenyatta National Hospital (KNH) surgical outpatient clinic for core needle biopsy for immunohistochemistry. The study was conducted following an ethical approval from the Kenyatta National Hospital-University of Nairobi (KNH-UON) ethics and research committee. Briefly, 4-5 passes were made into the breast mass of already consented newly diagnosed breast cancer patients using a 23-inch needle until FNA material was seen in the needle. The needle was removed and 6-8 core biopsies taken from the same mass using a core needle. The needle was then attached to a 10ml syringe and the material released onto a grease free glass slide. Another slide was used to...
spread the material thus obtaining a thin conventional smear, which was fixed in 95% alcohol immediately. On arrival in the laboratory the FNAC smear was stained using both papanicolaou staining and haematoxylin and eosin (H&E) methods for cytomorphological diagnosis of breast cancer. The remaining FNA material was aspirated with normal saline and the syringe contents released into a centrifuge tube which was then spun at 10000RPM for 5mins. The supernatant was discarded and the deposit used to prepare cellblocks whereby two drops of pooled plasma were added to the FNA deposit followed with gentle shaking after which two drops of thromboplastin were added and mixed well to activate the clotting factor. 2 drops of calcium ion were then added. The mixture was left to stand for 5 min to form a clot which was transferred to a moistened filter paper and wrapped well. The clot in the filter paper was placed in a cassette, and fixed in 10% neutral buffered formalin overnight. The sample was then processed using the routine processing method i.e. dehydration, clearing, impregnation and finally embedding to obtain quality cell block. Sectioning was done on both cell blocks and core biopsy and thin sections 3-5um placed on positively charged glass slides. Immunocytochemistry and immunohistochemistry staining were done on cell block sections and core biopsy sections respectively to assess HER2-Clone A0485 (DAKO, Glastrup, Denmark), ER-clone ID5 (DAKO, Glastrup, Denmark) and PR-clone PgR636 (DAKO, Glastrup, Denmark) protein expression using heat induced epitope retrieval. The stained sections were then mounted in DPX and placed on a clean dry surface and left to settle.

Results
The demographic information that was collected from the study respondents (n=39) included age, gender, occupation, parity, family planning method used, level of education and history of cervical cancer. The participants were aged between 21-85 years with a mean age of 48.26 (SD12.33) years, the median age was 46 years. The majority of the respondents 14(35.9%) were aged between 41to 50years. Out of the 39 respondents, 38 (97.4%) were female and 1 (2.6%) was male. The majority of the respondents 29 (74.4%) had cancer on their left breast. Most of the study respondents 23 (59%) were not using any family planning method. 26 (66.7%) had primary school education and 15 (38.5%) had secondary school education. 8 (20.5%) had breast cancer history either directly (having had breast cancer before) or indirectly (had a relative who had breast cancer). Breast cancer identification by the use of fine needle aspirate showed that majority of study participants 31(79.5) had ductal carcinoma. Core needle biopsy results based on histological grading, 17(43.6%) had ductal carcinoma grade II, 10 (25.6%) had ductal carcinoma grade III, 8 (20.5%) had ductal carcinoma grade I. 3 (7.7%) had invasive ductal carcinoma and 1(2.6%) had carcinoma with medullary features. Breast cancer classification based on IHC and ICC for ER, PR, and HER2 profile yielded the following results. Luminal A profile tumours (ER+, PR+, HER2-) had 7 (17.9%) on cell block and 10 (25.6%) on tissue block. Luminal B profile tumours (ER+, PR+, HER2+) had 8 (20.5%) on cell blocks and 9 (23.1%) on tissue blocks. HER2 over-expression (HER2+, ER-,PR-) consisted of 1(2.6%) on cell blocks and 2(5.1%) on histology blocks. The triple negative subtype was the most common having 17(43.6%) and 16(41.0%) on cell blocks and tissue blocks respectively. Cohen’s kappa was done to check the level of agreement between fine needle aspirate and core biopsy for immunohistochemistry.

On comparing immunocytochemistry and immunohistochemistry, for ER there was a concordance of 32/35(91.4%) r=0.842 Sensitivity of 83.3% and specificity of 85.0%. For PR the concordance was 32/35(91.4%) r=0.842 with sensitivity of 84.2%and specificity of 84.2%. For HER2 the concordance was 34/35 (97.1%) r=0.925 with a sensitivity of 88.9% and a
specificity of 96.3%. There was moderate agreement between the two methods, $k=0.719$, $p<0.001$

Breast cancer subtypes

The study findings showed that majority of the study participants, 17 (43.6) for cell block and 16 (41.0) for histopathology, had triple negative breast cancer subtype.

<table>
<thead>
<tr>
<th>Breast cancer Cases subtypes</th>
<th>Cell block</th>
<th>Histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Luminal A (ER+, PR+, HER2-)</td>
<td>7</td>
<td>17.9</td>
</tr>
<tr>
<td>Luminal B (ER+, PR+, HER2+)</td>
<td>8</td>
<td>20.5</td>
</tr>
<tr>
<td>HER2 Positive (HER2+, ER-, PR-)</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Triple Negative (HER2-, ER-, PR-)</td>
<td>17</td>
<td>43.6</td>
</tr>
</tbody>
</table>

ER - estrogen receptor; PR - progesterone receptor; HER2 - human epithelial growth factor receptor 2; $n$ - Observed count; (%) – Percentage

Table 1: Classification of Breast cancer subtypes

<table>
<thead>
<tr>
<th>Cell Block</th>
<th>Histopathology</th>
<th>Number of cases</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+</td>
<td>ER+</td>
<td>15</td>
<td>Concordant 32/35 (91.4%) $r=0.842$ Sensitivity 83.3%</td>
</tr>
<tr>
<td>ER-</td>
<td>ER-</td>
<td>17</td>
<td>Discordant 3/35 (8.6%) Specificity 85.0%</td>
</tr>
<tr>
<td>ER+</td>
<td>ER-</td>
<td>0</td>
<td>Discordant 32/35 (91.4%) $r=0.842$ Sensitivity 84.2%</td>
</tr>
<tr>
<td>PR+</td>
<td>PR+</td>
<td>16</td>
<td>Concordant 32/35 (91.4%) $r=0.842$ Sensitivity 84.2%</td>
</tr>
<tr>
<td>PR-</td>
<td>PR-</td>
<td>16</td>
<td>Discordant 3/35 (8.6%) Specificity 84.2%</td>
</tr>
<tr>
<td>PR+</td>
<td>PR+</td>
<td>0</td>
<td>Discordant 32/35 (91.4%) $r=0.842$ Sensitivity 84.2%</td>
</tr>
<tr>
<td>PR-</td>
<td>PR+</td>
<td>3</td>
<td>Discordant 3/35 (8.6%) Specificity 84.2%</td>
</tr>
<tr>
<td>HER2+</td>
<td>HER2+</td>
<td>8</td>
<td>Discordant 32/35 (91.4%) $r=0.842$ Sensitivity 84.2%</td>
</tr>
<tr>
<td>HER2-</td>
<td>HER2-</td>
<td>26</td>
<td>Discordant 3/35 (8.6%) Specificity 84.2%</td>
</tr>
<tr>
<td>HER2+</td>
<td>HER2-</td>
<td>0</td>
<td>Discordant 3/35 (8.6%) Specificity 84.2%</td>
</tr>
<tr>
<td>HER2-</td>
<td>HER2-</td>
<td>1</td>
<td>Discordant 3/35 (8.6%) Specificity 84.2%</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; PR, progesterone receptor; HER2, human epithelial growth factor receptor 2; $r$, correlation coefficient

Table 2: Comparison of ER, PR and HER2 staining on cellblock versus histopathology ($n=35$)

(a) FNA cell block ER strongly positive (b) tissue block ER strongly positive (c) FNA cell block HER2 strongly positive (d) tissue block HER2 positive (e) tissue block PR strongly positive (f) FNA cell block PR strongly positive
Discussion

Fine needle aspiration cytology has widely been used as part of the assessment for breast cancer in patients with palpable breast masses suspected to be cancerous. When an FNA report is positive for breast cancer, the patients are normally subjected to core biopsy procedure for histology to confirm the breast cancer and subsequent immunohistochemistry for hormonal receptors ER and PR as well as for human epidermal growth factor receptor 2 HER2/neu. The core biopsy procedure has some advantages in that it is very expensive compared to FNA and exposes the patient to a more invasive and painful procedure that has to be done under local anaesthesia. It would be therefore sensible if both cancer diagnosis and immunostaining to determine HER2, ER and PR status would be done using FNA material. The current study has demonstrated that FNAC can be used to determine PR, ER and HER2 expression patterns. The study had ER positivity of 42.9% on cellblocks and 51.3% on core needle biopsy which highly correlates with many Indian studies that have reported an ER positivity ranging from 40% to 45%. PR positivity was 45.7% on cell blocks and 53.8% on core needle biopsy. This was very close to results obtained by Kyama et al\textsuperscript{17} on the positivity of PR on cell blocks which was 47%. In the present study, HER2 positivity was 22.9% on cell blocks and 28.2% on core needle biopsy and this highly compared to Bird et al\textsuperscript{12} report of HER2/neu overexpression in 26% of breast cancer cases among Kenyan women.

When a correlation coefficient was done for cell block immunocytochemistry and tissue block immunohistochemistry, the results had a concordance of 32/35(91.4%) r=0.842 Sensitivity of 83.3% and specificity of 85.0% for ER. For PR the concordance was 32/35(91.4%) r=0.842 with sensitivity of 84.2% and specificity of 84.2%. For HER2 the concordance was 34/35 (97.1%) r=0.925 with a sensitivity of 88.9% and a specificity of 96.3%. Kumar et al did a similar study in 2012 and reported a concordance between cell blocks and tissue blocks of 90% for ER, 94% for PR and 90% for HER2\textsuperscript{16}. Their results were very similar to our study findings and this means that the two methods highly correlates with each other. Nishimura et al did a study on HER2 immunochemistry on cell blocks and tissue sections in 2016 and reported a concordance of 77% between the two methods \textsuperscript{18}. That was slightly lower compared to the results obtained in the present study of HER2 concordance of 97.1%. The Cohen’s kappa test was 0.719 indicating that there was an agreement between the two methods ICC and IHC. Most of the IHC and ICC results were concordant and many reasons would have attributed to the discordant results including but not limited to; low FNA cellularity, poor or inadequate fixation, inadequate antigen retrieval and errors in methodology. In the present study, the Allred scoring system was used to determine the ER/PR status whereby positivity in more than 10% of the tumor cells was considered to be positive. For HER2/neu, the grading for positivity was based on both proportion score and intensity in staining and a score of more than 3+ was considered positive. Previously paraffin embedded tissue sections IHC has been the method of choice for assessing ER, PR and HER2/neu status in patients with breast carcinoma. The present study perfectly agrees with the recent studies on assessment of HER2/neu, PR and ER expression patterns on cell blocks \textsuperscript{15, 16, 17}. Based on the study outcome, we concluded that cell blocks prepared from FNA can be adequately used to assess HER2/neu and hormonal receptor expression patterns in patients with breast cancer provided that the FNA contains enough tumor cells (high cellularity). This would be a very feasible method especially in developing countries like Kenya with limited resources. Most of the study respondents were unemployed house wives from rural areas and had primary school education indicating they were either not informed on breast cancer awareness programs or they had limited resources to access breast cancer screening. For most of them, the breast carcinoma had already advanced.
at the time of screening. It’s therefore recommended that routine breast cancer screening and assessment of protein expression patterns using FNAC be made available, accessible and affordable to all for better management of breast cancer cases. Programs on breast cancer awareness should be made accessible to all and especially to individuals with little or no education to make them understand the risk factors of breast cancer and the initial symptoms of breast cancer to facilitate early diagnosis and proper management of breast cancer cases. The triple negative subtype was found to be the most common (43.6%) on cell block and (41.0%) on tissue blocks. In as much as it seems to be the most difficult type to manage, the healthcare providers should be prepared on how to manage this subtype of cancer effectively. The study concluded that assessment of HER2, ER and PR on FNA cell blocks is a valid method since it is cheap, less invasive, time conscious and highly correlates with results obtained from tissue blocks. Therefore there is no point of taking tissue biopsy from cancer patients for the sole purpose of assessing ER, PR and HER2.

Acknowledgement and appreciation
Our appreciations go to the medical team and support staffs at the Kenyatta National Hospital (KNH) surgical out-patient clinic (SOPC) for the assistance accorded to us during recruitment of study subjects and sample collection. We also acknowledge the staffs at the KNH laboratory medicine department Histopathology and Cytopathology sections for allowing us to process our samples in the two laboratories and assisting where possible. As the corresponding author, my sincere appreciations go to one Dr Okemwa Pinda and his entire team for all their support throughout the study. I also acknowledge Mrs Rioki Josephine for the help and pieces of advice during my study. This work was partially supported by AFRICA-ai-JAPAN innovation research grant.

References


18. Nishimura R et al. HER 2 immunohistochemistry for breast cancer cell blocks can be used in the same way as that used for histological specimen. Diagnostic cytopathology Jan 2016 10: 274-279