Assessment of the Reliability of Immunocytochemical Detection of Estrogen and Progesterone Receptors Status on the Cytological Aspirates of Breast Carcinoma

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ABSTRACT

Purpose: Evaluation of the reliability of immunocytochemical staining for estrogen and progesterone receptor status on previously papanicolaou-stained fine needle aspiration smears of breast carcinoma cases.

Patients and Methods: This is a retrospective study conducted on destained smears of fine needle aspirates (FNA) obtained from 90 breast carcinoma cases. These cases underwent subsequent tumor resection and immunohistochemical detection of estrogen and progesterone receptors allowing a comparison between the immunocytochemical and immunohistochemical results (Gold Standard). Hypocellular slides were excluded from the current study. Only the nuclear staining was considered specific. The results were scored on the basis of the percentage of the positive nuclei among the total epithelial malignant cells after examination of the entire slide. Smears were interpreted as positive if ≥10% of the examined cells demonstrated nuclear staining. These results were then compared with the immunohistochemical results.

Results: For estrogen receptor immunocytochemistry, the overall cyto-histologic accuracy was 91.1% (82/90) while the discordance rate was 8.9% (8/90). The diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were 93%, 84.2%, 95.7%, and 76.2% respectively. For progesterone receptor immunocytochemistry, the overall cyto-histologic accuracy was 88.9% (80/90) while the discordance rate was 11.1% (10/90). The diagnostic sensitivity, specificity, positive predictive value, and negative predictive value were 87.1%, 95%, 98.4%, and 67.9% respectively.

Conclusion: Immunocytochemistry is considered as an efficient tool in evaluating estrogen and progesterone receptor status in breast carcinoma. The application of estrogen and progesterone receptor immunocytochemistry on previously Papanicolaou-stained slides provides an overall accuracy of 91.1% for estrogen receptor and 88.9% for progesterone receptor when compared with the immunohistochemical results.

Key Words: Estrogen receptor – Progesterone receptor – Breast carcinoma – FNA – Immunocytochemistry.

INTRODUCTION

Breast carcinoma is a common malignancy in women; it is the second leading cause of cancer-related death and the third most common cancer throughout the world [1]. Its incidence is rising in the world due to widespread awareness in the general population, especially about breast pathologies, and better diagnostic aids to detect the lesion at an early stage. The most accepted protocol followed for diagnosis of breast lumps is “Triple assessment” which includes clinical assessment, radiological assessment and cytopathological diagnosis [2]. Fine needle aspiration cytology of breast lumps is non-traumatic, accurate, easy, reliable, repeatable, and simple diagnostic test. It is also used for the avoidance of more invasive procedures. The behavior of breast carcinoma and its response to treatment can be predicted by various biological and molecular factors. These factors can be assessed on the cytological aspirates [3].

Traditional factor such as detection of the presence or absence of estrogen and progesterone receptors in breast carcinoma is known to be clinically essential for determining the responsiveness to the endocrine therapy and predicting prognosis in newly diagnosed and relapsed breast carcinoma [4]. High level of reliability, reproducibility, and simplicity are required for estrogen and progesterone receptors detection. The conventional methods of the receptors detection, such as the dextran-coated charcoal (DCC) assay and enzyme immunoassay (EIA),
are complicated biochemical methods that can not be conducted as routine tests in all clinical laboratories [2]. Furthermore, a tissue sample of 500mg is necessary for these assays. In contrast, immunocytochemistry and immunohistochemistry are simple and rapid methods routinely performed in pathologic laboratories. Since non-tumorous tissues may be included in the sample for the biochemical assays, the proportion of hormone receptors positive cells in the tumor can not be detected. In immunocytochemical and immunohistochemical methods, since cytolological and histological morphology can be observed, non tumorous tissue can be excluded from the receptor detection and only the proportion of receptor positive cells can be determined [1].

In locally advanced breast lesions, Tru cut biopsy is used to localize the hormone receptors by immunohistochemical markers in order to give the patient the preoperative chemotherapy [3]. But fine needle aspiration cytology is usually the initial method for the diagnosis of breast carcinoma, so it would save time if these markers are performed on cytological material at the time of diagnosis using immunocytochemistry [5]. Also it is sometimes very hard to obtain a tumor sample in cases of nipple discharge and Paget's disease; in which hormone receptors can not be assessed by the standard method; however, cytological specimens can be obtained from such cases and hormone receptors assessment can be performed on them [3]. Moreover, hormone receptors detection can be employed readily in patients with recurrent or metastatic breast cancer cases for whom surgery would otherwise not be indicated. In these cases, the determination of the hormone receptors status on cytological specimens can be extremely helpful and in some instances is mandatory [6]. Immunocytochemistry on fine needle aspiration materials has other potential application. Sequential determinations can be performed at different times during the course of systemic therapy, allowing direct monitoring of cellular modification that occurs within the tumor [7]. Also formalin fixative that is used for histology may destroy some epitopes in paraffin-embedded tissue while alcohol fixative may have a minimal effect on tumor cell antigenicity; thus fine needle aspiration cytology is considered as a reliable method to select patients for endocrine therapy [8]. Assessment of the hormone receptors status in cytological smears may also be a useful diagnostic adjunct in the evaluation of metastatic tumors of unknown origin [9].

The objective of the current study was to evaluate the reliability of the immunocytochemical method for estrogen and progesterone hormone receptor detection in breast carcinoma aspirates by comparing the results with those obtained by immunohistochemistry of the corresponding excised tissue sections (Gold standard).

**PATIENTS AND METHODS**

This study was retrospectively conducted on 90 modified Papanicolaou stained samples of breast carcinoma cases, which were used for routine cytologic diagnosis, at Cytology Unit, Pathology Department, National Cancer Institute, Cairo University during the year 2009. All these cases underwent subsequent tumor resection or biopsy as well as immunohistochemical detection of estrogen and progesterone hormone receptors allowing a comparison between the immunocytochemistry and the immunohistochemistry.

The most two representative Papanicolaou-stained slides were chosen for the immunocytochemical staining. Hypocellular smears slides were excluded from the current study.

The slides were destained using the technique described by Miller and Kubier [10]. The destained slides were subjected to immunocytochemical staining for estrogen and progesterone receptors according to the streptavidin-biotin-peroxidase technique using the rabbit monoclonal antibodies from manufacturer Thermo Scientific, U.K. Estrogen receptor (ER) mAb (clone; SP1), Progesterone receptor (PR) mAb (clone; SP2). The immunocytochemical staining allowed visualization of the target cell antigen, ER and PR. This technique involves the sequential incubation of the smear with unconjugated primary antibody specific to the target antigen, ER and PR. This technique involves the sequential incubation of the smear with unconjugated primary antibody specific to the target antigen, followed by the application of biotinylated secondary antibody that reacts with the primary antibody. Visualization is based on enzymatic conversion of a chromogenic substrate 3,3’ Diaminobenzidine (DAB) into a colored brown precipitate by horseradish peroxidase at the site of antigen localization [11].

Histologic sections of invasive duct carcinoma of known positive estrogen and progest-
Estrogen receptors reactivity were used as positive control, and a negative control was used by substituting Phosphate Buffer Saline (PBS) for the primary antibody. All controls yielded appropriate results. This means that the positive control slides showed strong positivity (3+) for ER and BR, thus it makes sure that the procedure was optimized and all reagents were working properly therefore any negative results were valid. While the negative control slides showed no staining, thus it helped to check the non-specific binding and the false positive results.

Only the nuclear immunoreactivity for hormone receptors was considered specific. Cytoplasmic and membranous staining was considered nonspecific. Both authors independently evaluated the presence of hormone receptors positive cells. The evaluation of the stained cells was done blindly, without knowledge of the histopathological hormonal receptors status of the correlated excised specimens.

All the epithelial malignant cells were examined on each slide for the presence of nuclear staining for estrogen and progesterone receptors. The results of immunoreactivity were scored semiquantitatively on the basis of the percentage of the positive nuclei among the total epithelial tumors after examination of the entire slide. Smears were interpreted as positive if ≥10% of the cells demonstrated nuclear staining. All cases were scored in 3 groups; 1+ (weak positive) if 10%-33% of the examined cells are stained positive, 2+ (moderate) if 33%-66% of the examined cells are stained positive, and 3+ (strong) if >66% of the examined cells are stained positive.

Results of the immunocytochemistry were compared with those obtained by immunohistochemistry of the tissue sections of the same patients. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated on the basis that the histological results were the gold standard. All these values were compared with other studies.

RESULTS

Seventy two cases (80%) of the 90 studied cases were proved histologically to be invasive duct carcinoma (NOS) of different grades, 9 cases (10%) were mixed infiltrating duct and lobular carcinoma, 6 cases (6.7%) were lobular carcinoma, 2 cases (2.2%) were papillary carcinoma, and one case (1.1%) was mucinous carcinoma.

Faint cytoplasmic staining for estrogen receptor was detected in 17 cases (18.9%), which did not cause interpretation problem and considered as non specific reaction. Faint to moderate background staining was detected in most cases which did not cause interpretation problem.

Immunocytochemical results:

Of the 90 histologically confirmed studied malignant cases, the total number of estrogen receptor positive cases was 69 cases (76.7%). The total number of progesterone receptor positive cases were 62 cases (68.9%), (Table 1).

Of the 69 estrogen receptor positive cases, 21 cases (30.5%) stained strongly (3+) (Fig. 1), 43 cases (62.3%) stained moderately (2+) (Fig. 3), and 5 cases (7.2%) stained weakly (1+). Among the 62 progesterone receptor positive cases, 11 cases (17.7%) stained strongly (3+) (Fig. 2), 51 cases (82.3%) stained moderately (2+) (Fig. 4), and no cases stained weakly (Table 2).

Compared to the immunohistochemistry, the diagnostic sensitivity, specificity, PPV, and NPV of estrogen receptor immunocytochemical staining were 93%, 84.2%, 95.7%, and 76.2% respectively. The overall accuracy was 91.1% (82/90) while the discordance rate was 8.9% (8/90) (Table 6).

Among the 62 progesterone receptor immunocytochemical positive cases, 61 cases (98.4%) were positive by immunohistochemistry (true
positive) and 1 case (1.6%) was negative by immunohistochemistry (false positive). Among the 28 progesterone receptor negative cases, 19 cases (67.9%) were negative by immunohistochemistry (true negative) and 9 cases (32.1%) were positive by immunohistochemistry (false negative) (Table 4).

In comparison to the immunohistochemistry, the diagnostic sensitivity, specificity, PPV, and NPV of progesterone receptor immunocytochemical staining were 87.1%, 95%, 98.4%, and 67.9% respectively. The overall accuracy was 88.9% (80/90) while the discordance rate was 11.1% (10/90) (Table 6).

There was disagreement between the results of immunocytochemistry and immunohistochemistry in 8 cases (8.9%) for estrogen receptor, 5 false negative cases and 3 false positive cases (Table 3). Three of the false negative cases were proved histologically to be invasive duct carcinoma grade 2 and 3 and the remaining two cases were mixed infiltrating duct/lobular carcinoma. As regard the 3 false positive cases, two cases were proved histologically to be invasive duct carcinoma grade 2 and one case was mucinous carcinoma (Table 5).

Regarding the progesterone receptor immunocytochemistry, there was disagreement with the immunohistochemistry in 10 cases (11.1%), 9 false negative cases and one false positive case (Table 4). Three of the false negative cases were also showing false negative results for the estrogen receptor staining. Two of them were invasive duct carcinoma grade 2 and one case was mixed infiltrating duct and lobular carcinoma on histopathologic diagnosis. The remaining 6 progesterone receptor false negative cases showed histopathologic diagnoses of invasive duct carcinoma grade 2 (3 cases), lobular carcinoma (2 cases), and papillary carcinoma (one case). The only progesterone receptor false positive case was proved histologically to be papillary carcinoma (Table 5).

### Table (1): Detection of estrogen and progesterone receptors by immunocytochemical staining in breast carcinoma patients under study.

<table>
<thead>
<tr>
<th></th>
<th>Positive (≥10% of tumor cells)</th>
<th>Negative (&lt;10% of tumor cells)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>69 (76.7%)</td>
<td>21 (23.3%)</td>
<td>90</td>
</tr>
<tr>
<td>PR</td>
<td>62 (68.9%)</td>
<td>28 (31.1%)</td>
<td>90</td>
</tr>
</tbody>
</table>

ER: Estrogen receptors. PR: Progesterone receptors.

### Table (2): The positivity rate (scores) of the hormone receptors status of the positive cases by immunocytochemical staining in breast carcinoma patients under study.

<table>
<thead>
<tr>
<th></th>
<th>Weak 1+ (10%-33%)</th>
<th>Moderate 2+ (33%-66%)</th>
<th>Marked 3+ (&gt;66%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>5 (7.2%)</td>
<td>43 (62.3%)</td>
<td>21 (30.5%)</td>
<td>69</td>
</tr>
<tr>
<td>PR</td>
<td>0</td>
<td>51 (82.3%)</td>
<td>11 (17.7%)</td>
<td>62</td>
</tr>
</tbody>
</table>

ER: Estrogen receptors. PR: Progesterone receptors.

### Table (3): Comparison of estrogen receptor detection by immunocytochemistry and immunohistochemistry in breast carcinoma patients under study.

<table>
<thead>
<tr>
<th>IHC results</th>
<th>ER-ICC results</th>
<th>PR-ICC results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n=69)</td>
<td>Negative (n=21)</td>
</tr>
<tr>
<td>Positive</td>
<td>66 (95.7%) (TP)</td>
<td>5 (23.8%) (FN)</td>
</tr>
<tr>
<td>Negative</td>
<td>3 (4.3%) (FP)</td>
<td>16 (76.2%) (TN)</td>
</tr>
</tbody>
</table>

ICC: Immunocytochemistry. IHC: Immunohistochemistry. ER: Estrogen receptors. TP: True positive cases. FN: False negative cases. FN: False positive cases. TN: True negative cases.

### Table (4): Comparison of progesterone receptor detection by immunocytochemistry and immunohistochemistry in breast carcinoma patients under study.

<table>
<thead>
<tr>
<th>IHC results</th>
<th>ER-ICC results</th>
<th>PR-ICC results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n=62)</td>
<td>Negative (n=28)</td>
</tr>
<tr>
<td>Positive</td>
<td>61 (98.4%) (TP)</td>
<td>9 (32.1%) (FN)</td>
</tr>
<tr>
<td>Negative</td>
<td>1 (1.6%) (FP)</td>
<td>19 (67.9%) (TN)</td>
</tr>
</tbody>
</table>

ICC: Immunocytochemistry. IHC: Immunohistochemistry. ER: Estrogen receptors. TP: True positive cases. FN: False negative cases. FP: False positive cases. TN: True negative cases.

### Table (5): Immunocytochemical hormone receptors of discordant cases in relation to the histopathologic diagnoses in breast carcinoma patients under study.

<table>
<thead>
<tr>
<th>Histopathologic diagnoses</th>
<th>ICC discordant cases of ER</th>
<th>ICC discordant cases of PR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FN</td>
<td>FP</td>
</tr>
<tr>
<td>Invasive duct carcinoma</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mixed D/L carcinoma</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Lobular carcinoma</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Papillary carcinoma</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mucinous carcinoma</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

ER: Estrogen receptors. PR: Progesterone receptors. ICC: Immunocytochemistry. TP: True positive cases. FN: False negative cases.
Table (6): Diagnostic reliability of immunocytochemical staining of estrogen and progesterone receptors compared to immunohistochemistry.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ER (%)</th>
<th>PR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sensitivity</td>
<td>93.0</td>
<td>87.1</td>
</tr>
<tr>
<td>specificity</td>
<td>84.2</td>
<td>95.0</td>
</tr>
<tr>
<td>PPV</td>
<td>95.7</td>
<td>98.4</td>
</tr>
<tr>
<td>NPV</td>
<td>76.2</td>
<td>67.9</td>
</tr>
<tr>
<td>Accuracy</td>
<td>91.1</td>
<td>88.9</td>
</tr>
<tr>
<td>Discordance</td>
<td>8.9</td>
<td>11.1</td>
</tr>
</tbody>
</table>

ER: Estrogen receptors.  
PR: Progesterone receptors.  
PPV: Positive predictive value.  
NPV: Negative predictive value.

Fig. (1): Fine needle aspirate from a case of breast carcinoma showing positive nuclear staining for estrogen receptor, score 3+ (Avidin-biotin-peroxidase x100).

Fig. (2): Fine needle aspirate from a case of breast carcinoma showing positive nuclear staining for progesterone receptor, score 3+ (Avidin-biotin-peroxidase x400).

Fig. (3): Fine needle aspirate from a case of breast carcinoma showing positive nuclear staining for estrogen receptor, score 2+ (Avidin-biotin-peroxidase x400).

Fig. (4): Fine needle aspirate from a case of breast carcinoma showing positive nuclear staining for progesterone receptor, score 2+ (Avidin-biotin-peroxidase x400).

Fig. (5): Fine needle aspirate from a case of breast carcinoma showing negative nuclear staining for estrogen receptor (Avidin-biotin-peroxidase x400).
DISCUSSION

Recently the possibility of using cytological materials, not only for morphological diagnosis but also for biologic characterization of the tumors, has added an advantage to this technique [12]. Fine needle aspiration cytology has been proved to be an important patient-friendly procedure in breast lumps reducing the patient anxiety in many situations [13]. Several methods were developed for in-vitro quantitative measurement of hormone receptor status including sucrose density gradient, dextran-coated charcoal (DCC) assay, gel electrophoresis, hydroxylapatite, and gel infiltration assay [14]. The production of monoclonal antibodies against estrogen and progesterone receptors protein have permitted the development of estrogen and progesterone receptors assessment based on direct antigenic recognition rather than steroid binding activity of the biochemical assays. These monoclonal antibodies are highly specific and sensitive for the detection and quantification of hormone receptor status in human breast carcinomas [15].

The objective of the current study was to compare immunocytochemical expression of estrogen and progesterone receptors on destained Papanicolaou smears, obtained by preoperative fine needle aspiration cytology from primary breast carcinoma, with immunohistochemistry results obtained from the corresponding surgical excisions.

Many previous studies have compared the hormone receptor status on cytological imprint obtained at breast surgery or fine needle aspiration cytology from surgical specimens with the corresponding value obtained from the biochemical assays of the respective surgical biopsies. Most of these studies showed a good correlation between all techniques [1,3]. Relatively few studies have investigated the correlation between hormone receptor assessment by immunocytochemistry on preoperative fine needle aspiration cytology with the results determined by immunohistochemistry of the corresponding surgical samples [5,7,16].

Some authors evaluated the hormone receptor status using immunocytochemical technique on archival Papanicolaou stained fine needle aspiration slides and concluded that Papanico-

laou-stained slides used as such, without destaining, for immunocytochemistry showed slightly better agreement with immunohistochemistry than the destained slides [17]. While others reported that using Papanicolaou-stained slides for immunocytochemistry without destaining but with antigen retrieval showed a much better agreement with immunohistochemistry than destained slides. They explained that by loss of some receptor antigenicity by the harsh destaining procedure [9]. However most other authors assessed the hormone receptor status on cytological smears using destained slides or unstained slides and achieved good results [4,18]. For these, we chose the destained procedure which is the most common one.

We assumed 10% of the stained cells for estrogen and progesterone receptors immunocytochemical staining as a cut-off value in order to distinguish between receptors positives and negatives. Masood, [14] in her study on the imprint cytologic preparation obtained at breast surgery considered that the tumors were positively stained if ≥20% of the tumor cells showed nuclear staining. However many other authors concluded that lowering the cut-off point to 10% for reporting hormone receptors will help in reducing the false negative results in breast cancers [5,17,19].

In the current study, 17 cases (18.9%) showed faint cytoplasmic staining for estrogen receptors, that did not cause interpretation problems and it was considered as non specific reaction. Kumar et al., [20] in their study concluded that naturally occurring short form of the metastatic tumor antigen 1 (MTA1) sequesters estrogen receptor in the cytoplasm, which can be detected by using a sensitive monoclonal antibody, therefore, their cases with cytoplasmic reaction for estrogen receptors did not considered as non specific staining.

In the present study, the concordance results of both estrogen (91.1%) and progesterone receptor (88.9%) were in line with the concordance rate of 84% to 96% that reported in literature [21]. The concordance between cytology and histology were 84% for estrogen receptor and 90% for progesterone receptor in the study done by Malaviya et al., [22].

Nizzoli et al., [7] found 89% agreement for estrogen receptor immunocytochemistry and
78% for progesterone receptor when compared with the immunohistochemical results. Zoppi et al., [18] concluded that for estrogen and progesterone receptor immunocytochemistry, a cytointestinal correlation of 94% and 71.2% were found respectively with a sensitivity of 96.1% and specificity of 86.9% for estrogen receptor and 65.7% sensitivity and 83.3% specificity for progesterone receptor. Tafjord et al., [16] recorded 89% and 63% concordance rate for estrogen and progesterone receptors immunocytochemistry, respectively; these results were changed to 98% and 91% concordance rate respectively after re-evaluation of most slides by another pathologist’s team.

However our results were much higher than that reported by Radhika and Prayaga [5] who recorded 50% concordance rate for estrogen receptor and 29% concordance rate for progesterone receptor immunocytochemistry with 33% sensitivity and 75% specificity for estrogen receptor; and 25% sensitivity and 33% specificity for progesterone receptor immunocytochemistry. They attributed the cause of their poor results to technical factors such as using poor buffer and improper antigen retrieval due to financial cause. Although most authors recorded that the antigen retrieval is not a problem in immunocytochemistry [23]. Gong et al., [9] reported that using of antigen retrieval converted most of the hormone receptor negative smears, in their series on previously stained slides, to positive smears raising the final concordance rate (from 71.4% to 93%) and sensitivity (from 61.9% to 90.5%) near the level of the immunohistochemical results. They also concluded that antigen retrieval led to stronger staining intensity without causing false positive results.

In the present study, the 2 studied papillary carcinoma cases were falsely diagnosed on progesterone receptor immunocytochemistry, one case was false positive and one case was false negative. The only studied mucinous carcinoma case was falsely diagnosed on estrogen receptor immunocytochemistry. Two cases out of the 6 studied lobular carcinoma cases were false negatively diagnosed on progesterone receptor immunocytochemistry. Two cases out of the 9 studied mixed infiltrating duct and lobular carcinoma cases were false negatively diagnosed on estrogen receptor immunocytochemistry (Table 5). Zoppi et al., [18] found that one case out of the 4 studied mucinous carcinoma cases were falsely diagnosed on progesterone receptor immunocytochemistry while 2 cases out of the 4 studied papillary carcinoma cases were falsely diagnosed on estrogen and progesterone receptor immunocytochemistry and 2 out of the 26 studied lobular carcinoma cases were falsely diagnosed on estrogen receptor immunocytochemistry. Nizzoli et al., [6] reported that in contrast to invasive duct carcinoma, all other studied histologic type, lobular, papillary, mucinous, and medullary, were truly positive for estrogen and progesterone receptor immunocytochemistry. Koyatsu et al., [1] found that all the falsely diagnosed cases, in their study, were invasive duct carcinoma, scirrhous carcinoma.

A similar discrepancy rate for estrogen receptor staining was observed by Nizzoli et al., [7] and Hanley et al., [8] who reported 11% and 12.2% discrepancy rate, respectively. However they recorded higher discrepancy rate of 21.6% and 26.9% for progesterone receptor staining, respectively. Nizzoli et al., [7] in their study attributed the cause of the discordant cases between the immunocytochemistry and immunohistochemistry to the alternation of the receptor molecules during the immunocytochemical procedure or due to the presence of tumor heterogeneity that in some instances could lead to non representative cytotologic sample. Tumor heterogeneity referred to the variation in the percentage of positive cells in different areas of the same tumor that often produced the effect of a zonal distribution of estrogen or progesterone receptor positive cancer cells. But fine needle aspiration cytology usually permits sampling of several areas of the same tumors and allows better assessment of the hormone receptors heterogeneity, in contrast to the histopathology [24]. Gong et al., [9] reported that tumor heterogeneity appeared to be an unlikely factor contributing to negative results in the cytology. They suggested that the exact cause of the false staining might be due to unknown preanalytic factors rather than tissue heterogeneity.

Nizzoli et al., [7] also attributed the discrepancies between the cytology and histology to the use of different monoclonal antibodies for immunocytochemical and immunohistochemical assays. Cano et al., [19] reported that the results obtained on fine needle aspiration smears and
histologic sections using two different monoclonal antibodies were quite similar. However, this explanation for the discrepancy between the cytological and histological results was avoided in the current study as we used the same monoclonal antibodies for both techniques. Percentages of immunostained cells above and below, but very close to, the cut off point may also account for the disagreement in their study.

Hanley et al., [8] explained their discordance rate between the cytology and the histology, 12.5% for estrogen receptors and 26.9% for progesterone receptors, to confounding factors such as bloody samples, paucicellular aspirates, using of alternative fixative, and sampling problems, which can alter antigen expression/detection and these can influence immunocytochemical preparation. They reported that these limitations have to be addressed and kept in mind before estrogen and progesterone receptor results from breast carcinoma fine needle aspirate are applied for treatment decisions. In the current study, we selected the most representative slides excluding the severe bloody and hypocellular slides as well as we used alcohol as a fixative.

Tafjord et al., [16] recorded 11% and 37% discrepancy rate for estrogen and progesterone receptor staining, respectively and concluded that 53% of the false results were caused by wrong interpretation of histologic findings, 10% by wrong interpretation of cytologic findings, 17% by sampling error, and 20% of cases were not available for re-evaluation by another pathologists team. They recommended the use of strict criteria for interpretation of cytologic results of the hormone receptor status as wrong interpretation was a far more frequent cause of clinically relevant discrepancies than sampling errors.

Some authors also concluded that the main cause of testing error were due to wrong interpretation of the results and lack of standardization for the immunocytochemistry between laboratories [4]. Cavaliere et al., [13] recommended that the assessment of the estrogen and progesterone receptors immunocytochemistry should be performed using computer assistance to avoid wrong interpretation that lead to false results and inter-institutional variations.

Erroneous results, 50% for estrogen receptor and 71% for progesterone receptor staining, obtained by Radhika and Prayaga [5] were explained by suboptimal assays due to the use of improper techniques in the form of using an improper antigen retrieval and using pressure cooker for heating instead of the microwave.

Krishnamurthy et al., [17] studied the hormone receptor assessment by immunocytochemistry on archival Papanicolaou stained smears and concluded that the negative results were less reliable as an indicator of the true hormone receptor status than the positive results. This result agreed with our study where most of discordant cases were among the negative results (Tables 3 & 4), 5 cases (28.8%) out of 21 negative estrogen receptor results and 9 cases (32.1%) out of the negative progesterone receptor results. While the positive results showed only 3 false positive results (4.3%) among the 69 estrogen positive cases and one false positive result (1.6%) among the 62 progesterone positive cases.

Conclusion:
The application of estrogen and progesterone receptor immunocytochemistry on previously Papanicolaou-stained slides provide overall accuracy of 91.1% for estrogen receptor and 88.9% for progesterone receptor when compared with the immunohistochemical results, hence showed that immunocytochemistry is considered as an efficient tool in evaluating estrogen and progesterone receptor status in breast carcinoma especially whenever surgical biopsy is not indicated or not possible and when information about estrogen and progesterone receptor status is required at the time of the clinical diagnosis.

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