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## Steroid hormone receptors, MIB-1, p53, and c-erb-B2 expression on breast cancer: Comparison of immunohistochemistry on cell block and fine needle aspiration and tissue sample, in northwest Iran

### *Abstract*

**Background:** Fine-needle aspiration of breast cancer often provides moderate cellular material that is representative of the tumors. These samples can be used not only for cytological diagnosis but also to obtain information on the prognosis and likely response to therapy by using immunohistochemical staining studies.

**Methods:** We assessed the degree of correlation between prognostic biologic markers by means immunohistochemistry (IHC) on cytoblock obtained from fine-needle aspiration (FNA) and immunohistochemical determination on their corresponding tissue sample of five markers; steroid hormone receptors, MIB-1 (Ki-67), p53, and c-erb-B-2 (Her2/neu) in 45 mastectomy of breast cancer.

**Results:** Interobserver reproducibility ranged from 93 to 100%, depending on the marker. A good correlation was observed between immunostaining assessment on cytoblock and on their corresponding tumor tissues as follows: Ki-67 (87%), ER (80%), PR (93%), p53 (96%), and c-erb-B-2 (76%).

**Conclusion:** We conclude that cytoblock prepared from fine-needle aspiration specimens of breast cancer is a useful and noninvasive procedure when planning neoadjuvant treatment.

**Keywords:** Breast cancer, Fine-needle aspiration, Immunohistochemistry, cell block, Esteroid receptors, P53, Ki67, C-erb-B2

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In some regions of the world, breast cancer mortality beginning to fall due to earlier diagnosis and improved therapy. The diagnosis of breast cancer is made by clinical examination, mammography, FNA (triple test), needle core biopsy and open biopsy. In several countries, mammographic screening allows the early diagnosis of tumors. However, when a tumor is suspected, morphological analysis alone can establish the diagnosis of carcinoma. In this context, guided fine-needle aspiration has become increasingly popular for obtaining tissue specimens for the diagnosis of malignant breast diseases. Major progress has been made in recent years in IHC detection of various markers (cell proliferation markers, hormone receptors, etc.), improving knowledge and management of breast cancer. The overexpressoin of P53 may itself be a prognostic factor in human breast cancer. Fine-needle aspiration, which is useful for pretherapeutic diagnosis of breast cancer and for monitoring its progression often, provides highly cellular material representative of the lesions which can be used for such analyses (1-5). Various studies have shown that nuclear and cytoplasm markers can be detected individually by means of ICC on aspiration smears, whereas, IHC studies of cells present in paraffin miniblocks can simultaneously identify several markers on consecutive sections (6).

Yadutsuoiverp eno nI reported that P53 overexpressoin on tissue paraffin block was not correlated with tumor size, tumor type, nodal status and side of involved breast (7). The aim of this study was to assess the reliability of immunohistochemical detection on cell block compare to IHC on their corresponding tissue for the detection of markers which considered the predictive of the prognosis and/or the response to therapy in cytoblock preparations of fine-needle aspiration of breast cancer specimens. In this study, we compared marker detection on cytoblocks and on the corresponding tissue samples to determine the reliability and difficulties of cytoblock assessment on the determination of estrogen receptors (ERs) and progesterone receptors (PRs), MIB-1 (Ki-67), p53, and c-erb-B-2 in breast cancer.

## Methods

Our type of study was a prospective diagnostic value (analytic-descriptive). FNA on a malignant breast tissue of 45 patients who were referred to Imam Reza Hospital during a period of 14 months (August 2008-October 2009) was studied. Five marker expressions on them by preparing cell blocks and IHC staining have been studied; in addition to IHC, staining on the tissue blocks of their corresponding surgical samples were evaluated. However, the FNA of breast cancer often provides highly cellular material that can not only be for cytologic diagnosis but also obtain information about prognosis and likely response to therapy. Cell block had been prepared for each specimen then IHC was done for 5 markers, meanwhile, their corresponding tissue block had been prepared and IHC was performed for all. Fine needle aspiration cytology (FNAC) of 45 primary breast cancers immunostained for ER and PR, MIB-1 (Ki-67), p53, and c-erb-B-2 during a period of 14 months, formed the material of the study. First Papanicolaou and Giemsa -stained slides were taken from all the specimens.

Formalin fixed samples were obtained from excisional biopsy of patients with breast carcinoma that had positive FNA for malignant cells existence before. For each patient, one block containing the central part of the tumor was selected for IHC.

In cell block preparation after fine-needle cytopuncture, each specimen was fixed in neutral alcohol formal for 16-24 hours according to Shandon kit's manufacturer's instructions (Shandon Inc., Pittsburgh, PA), putting the cellular material on lens paper and preparing a tissue cassette. Tissue cassettes

were put in tissue processor and finally in the prepared paraffin embedded cell blocks.

IHC examination on formalin fixed paraffin embedded blocks samples were used to determine the status of the biological markers and therefore, 2.5  $\mu$ m sections were cut for immunohistochemical staining. In the IHC test, the following monoclonal antibodies, each from Zymed laboratories Inc. were also obtained and used:

1. Monoclonal mouse anti ER, Clone: 1D5, Isotype: cellular IgG1-Kappa
2. Monoclonal mouse anti PR, Clone: PR-2C5, Isotype: IgG1-Kappa
3. Mouse antihuman phosphoprotein P53, clone: BP53.12, Isotype: IgG2a, Kappa
4. Monoclonal mouse anti-C-erbB2, Clone: CB11, Isotype: IgG
5. Monoclonal mouse anti-Ki67, Clone / PAD: 7311, Isotype IgG1

The immunostaining results interpreted by two pathologists using a light microscope.

**Immunohistochemistry:** Sections were mounted on triethylenethiophosphoramide- pre-coated slides and allowed to dry at 50° C overnight. The sections were then dewaxed in xylene and hydrated through graded concentrations of alcohol. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide for 15 min. The sections were then immersed in a thermo resistant plastic box containing 10 mL citrate buffer, pH 6.0, and treated in a microwave oven four times at 750 W for 5 min each. The sections were allowed to cool at room temperature for 30 min and were rinsed in tris-buffered saline. The blocking reagent was tipped away and the primary antibodies were added for one hour. A standard avidin-biotin-horseradish peroxidase complex was used to reveal antibody-antigen reactions. Staining was routinely developed using diaminobenzidine tetrahydrochloride followed with a light hematoxylin counter stain.

**Assessment of staining:** The percentage of positive cells was scored for all of the markers. At least 100 malignant cells were considered suitable for immunohistochemistry on cytoblocks. Immunoreactivity for ER and PR was graded as negative and positive according to more than 10% of tumor cells in high grade tumor and less than 10% of tumor cells (7). For HER-2/neu, tumors were considered positive when at least 10 percent of tumor cells had partial or complete membranous staining (8). For p53 and Ki67, cutoff scores of 10% of cells with nuclear staining (9).

All the tests were performed with the Stata View program (Abacus Concepts, Berkeley, California, USA). The analytical performance of IHC in cytological samples was also analyzed for specificity, sensitivity, and negative and positive predictive values.

## Results

In total, 45 cases were assessed by IHC on cytoblock and tissue specimens for the determination of estrogen receptors (ERs) and progesterone receptors (PRs), MIB-1 (Ki-67), p53, and c-erb-B-2 in breast cancer. Forty four patients (97.8%) were females and one patient was male (2.2%). The mean age of patients was 47 years range (20-67 yrs). Ten cases were in the mean age of 20 to 40 years, 30 patients were in the mean age of 41 to 60 years, 5 patients were in the mean age of more than 60 years old. There were three histopathological types of reports between our patients. About 82.2% of patients were diagnosed for invasive ductal carcinoma alone, 2.2% of patients were diagnosed for carcinoma in situ alone, and 15.6% of remaining patients were diagnosed for invasive ductal carcinoma with carcinoma in situ component. All patients expressed biological markers like steroid receptors, MIB-1 (Ki-67), p53, and c-erb-B2 equally and there is no significant difference between the three types of these in our patients.

Interobserver reproducibility (MB and VLD) on the cytoblocks was 93% for Ki-67, 100% for ER, 93% for PR and p53, and 89% for c-erb-B-2. The concordance rate in the three-group classifications was 96% (ER), 87% (PR), 84% (p53), 87% MIB-1 and 80% (c-erb-B-2).

We consider median ER percentages of positive nuclei about 60% (0 to 100%) on cytoblocks and 70% (0 to 100%) on tissue specimens. ER expression in IHC on tissue specimen was positive in 26 cases (57.8%) and negative in 19 cases (42.2%). Otherwise, ER expression in IHC on cytoblock specimen was positive in 17 cases (37.8%) and negative in 28 cases (62.2 %). The sensitivity of IHC stain on cytoblock for expressing ER marker was 65.4%, the specificity was 100%, the positive predictive value was 100% and the negative predictive value was 68%. All in all, the concordance rate between IHC stain on cytoblock and tissue specimen as a gold standard test was 80% (table 1, figure 1). We consider median PR percentages of positive nuclei were 20% (0 to 95%) on cytoblocks and 25% (0 to 100%) on tissue specimens. PR expression in IHC on tissue specimen

was positive in 16 cases (35.6%) and negative in 29 cases (64.4 %). Otherwise PR expression in IHC on cytoblock specimen was positive in 15 cases (33.3%) and negative in 30 cases (66.7%). The sensitivity of IHC stain on cytoblock for expressing PR marker was 87.5%, the specificity was 96.6%, the positive predictive value was 93% and the negative predictive value was 93%. All in all, the concordance rate between IHC stain on cytoblock (table 1, figure 2). We considered median p53 percentages of positive nuclei that were 15% (0 to 100%) on cytoblocks and 8% (0 to 100%) on tissue specimens. P53 expression in IHC on tissue specimen was positive in 20 cases (44.4%) and negative in 25 cases (55.6%). Otherwise, P53 expression in IHC on cytoblock specimen was positive in 18 cases (40.0%) and negative in 27 cases (60.0%). The sensitivity of IHC stain on cytoblock for expressing P53 marker was 90.0%, the specificity was 100%, the positive predictive value was 100% and the negative predictive value was 93%. All in all, the concordance rate between IHC stain on cytoblock and tissue specimen as a gold standard test was 96% (table 2, figure 3).

We considered median c-erb-B-2 percentages of positive cells that were 15% (0 to 100%) on cytoblocks and 40% (0 to 100%) on tissue specimens. C-erb-B2 expression in IHC on tissue specimen was positive in 30 cases (66.7%) and negative in 15 cases (33.3%). Otherwise, C-erb-B2 expression in IHC on cytoblock specimen was positive in 20 cases (44.4%) and negative in 25 cases (55.6%). The sensitivity of IHC stain on cytoblock for expressing C-erb-B2 marker was 73.3%, the specificity was 80%, the positive predictive value was 88% and the negative predictive value was 60%. All in all, the concordance rate between IHC stain on cytoblock and tissue specimen as a gold standard test was 76% (table 2, figure 4).

We considered median MIB-1 percentages of positive cells were that (0-100%) on cytoblocks and more than 10% (0 to 100%) on tissue specimens. MIB-1 expression in IHC on tissue specimen was positive in 37 cases (82.2%) and negative in 8 cases (17.8%). Otherwise, MIB-1 expression in IHC on cytoblock specimen was positive in 35 cases (77.8%) and negative in 10 cases (22.2%). The sensitivity of IHC stain on cytoblock for expressing MIB-1 marker was 89.2%, the specificity was 75%, the positive predictive value was 94% and the negative predictive value was 60%. All in all, the concordance rate between IHC stain on cytoblock and tissue specimen as a gold standard test was 87% (table 2, figure 5).

**Table 1: Estrogen Receptor; Progesterone Receptor, P53 oncogen, KI67 and Her2neu oncogen, Comparison between IHC results on cell block and tissue specimen of breast cancer**

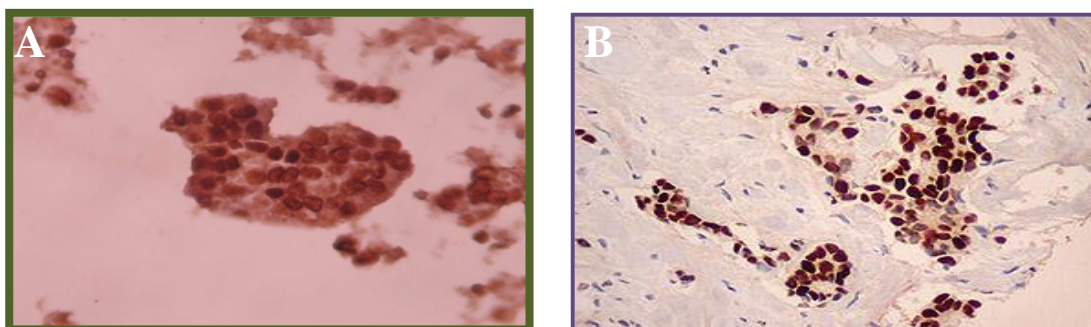
Markers	ER	PR	P53	MIB1	Ki 67
IHC-positive on Tissue & cell block	17	14	18	33	22
IHC-negative on Tissue& cell block	19	28	25	10	12
IHC-negative on tissue& positive on cell block	0	1	0	2	3
IHC-positive on tissue &negative on cell block	9	16	2	4	8
Total	45	45	45	45	45

**Table 2: Steroid receptors (ER, PR), Her2/neu, P53, Ki67; Comparison between IHC results on cell block and tissue specimen of breast cancer**

Marker	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Concordance	LR+	LR-
ER	65.4%	100%	100%	68%	80%	$\infty$	0
PR	87.5%	96.6%	93%	93%	93%	25.7	77.2
HER2/neu	73.3%	80%	88%	60%	76%	36.6	29.9
P53	90%	100%	100%	93%	96%	$\infty$	10
KI67	89.2%	75%	94%	60%	87%	35.6	23.2

**Table 3: Comparison of ER and PR results with other studies**

Marker	Concordance		PPV		NPV		Specificity		Sensitivity	
	PR	ER	PR	ER	PR	ER	PR	ER	PR	ER
Our study	93%	80%	93%	100%	93%	68%	96.6%	100%	87.5%	65.4%
Wijayanayaa et al	83%	83%	-	-	-	-	-	-	-	-
H Liu et al	82%	70%	100%	100%	73%	52%	100%	100%	-	-
M. Brifod et al	70.9%	70%	100%	100%	73%	52%	100%	100%	-	-

**Figure 1: A highly positive breast tumor for estrogen receptors on cell block (A) and on tumor tissue specimen (B) ( $\times 400$ ).**



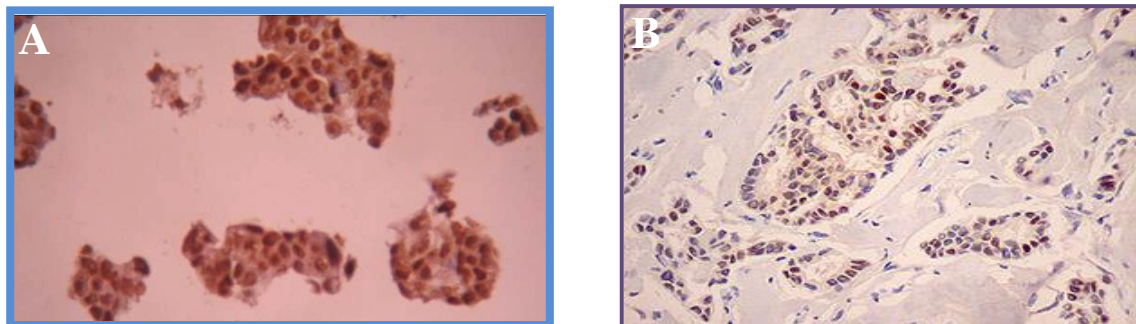


Figure 2: a highly positive breast tumor for Progesterone receptors on cell block (A) and on tumor tissue specimen (B) ( $\times 400$ ).

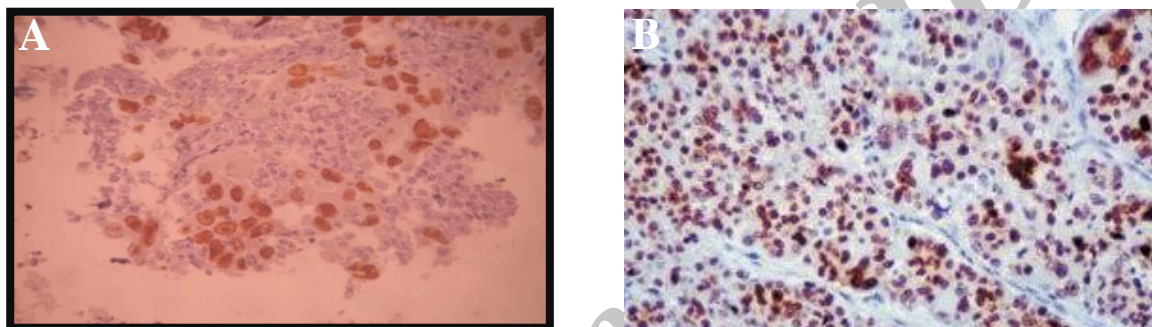


Figure 3: A highly positive breast tumor for P53 oncogene on cell block (A) and on tumor tissue specimen (B) ( $\times 400$ ).

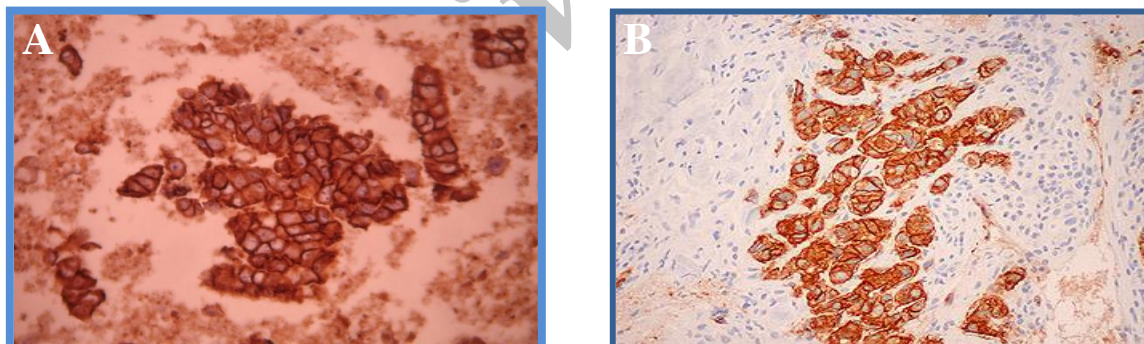


Figure 4: A highly positive breast tumor for C-erb-B2 oncogene on cell block (A) and on tumor tissue specimen (B) ( $\times 400$ ).

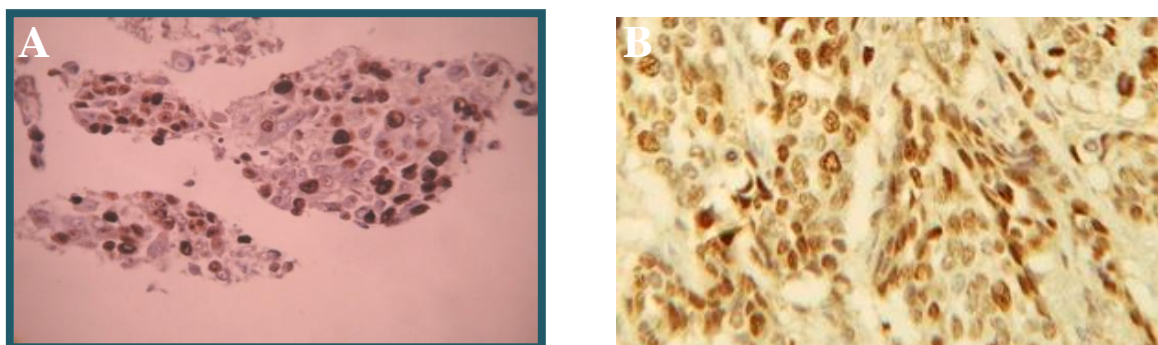


Figure 5: A highly positive breast tumor for MIB-1 oncogene on cell block (A) and on tumor tissue specimen (B) ( $\times 400$ ).

## Discussion

A fine needle biopsy is an effective tool in evaluating and diagnosing suspect lumps or masses. A quick diagnosis can mean that cancer is detected early, giving more options for treatment, or that benign lumps are diagnosed without the need for surgery. It is non-invasive and only slightly uncomfortable as compared to a surgical biopsy which requires a general anesthetic, involves pain and the possibility of infection or scarring. Fine needle aspiration biopsies do require some expertise to perform and interpret. To ensure that an accurate result is achieved, fine-needle aspiration of breast carcinoma often provides moderate cellular material that is representative of the tumors. These samples can be used not only for cytology diagnosis but also to obtain information on the prognosis and likely response to therapy.

The possibility of including cells in miniblocks and thereby obtaining multiple sections has been the subject of several reports. Makjoub et al. reported that P53 overexpression on tissue paraffin block was not correlated with tumor size, tumor type, nodal status and side of involved breast (7). Pinder et al. first studied the value of this method for analyzing multiple prognostic markers by means of IHC in breast cancer. They focused on samples obtained by fine-needle aspiration (FNA) of resected tumors, whereas we worked exclusively on the samples obtained before therapy by in vivo cytopuncture, when cellularity of the sample was obvious (6). Consequently, we did not seek to determine what proportion of breast cancers would yield useable cytoblocks. We used their technique with slight modifications. To evaluate the reliability of the method and to identify possible sources of error in the assessment of the different immunomarkers on cytoblocks of the primary breast tumor, we compared our breast cytoblock findings with those obtained on the corresponding tissue specimens. Overall, cytoblock immunostaining was of good quality and free of artifacts such as excessive background.

We tested a series of cytoblocks from 45 patients with primary breast cancer. We compared marker detection on cytoblocks and on the corresponding tissue samples to determine the reliability and difficulties of cytoblock assessment on determination of estrogen receptors (ERs) and progesterone receptors (PRs), MIB-1 (Ki-67), p53, and c-erb-B2 in breast cancer. Forty four patients (97.8%) were females and one male (2.2%). The mean age of the patients was 67 years (ranged 20-67 years). According to our study,

the mean age of breast cancer in western of Iran is lower than that reported in other parts of Iran which makes it more important our study (8-10). The concordance rate between ER expression of IHC stain on cytoblock and tissue specimen as a gold standard test was 80% which was similar from the findings of Liu et al. (11). The concordance between cytology and histology was 70% for ER. The positive predictive value of ER expression in IHC on cytoblock specimen in this study was 100% (ER expression in IHC on tissue specimen (11,12). Therefore, with positive ER expression in IHC on cytoblock specimen, practitioner can make candidate the patient for hormone therapy without doing more invasive procedure (Table 2).

The concordance rate between PR expression of IHC stain on cytoblock and tissue specimen as a gold standard test was 93%. Whereas in one study concordance rate was 83% and 70.9% (12). In Briffod et al. study, the concordance between cytology and histology was 84% for PR in the study (13). Our finding with the results of other studies confirmed that positive PR marker together with positive ER marker makes better answer to hormone therapy treatment (Table 3).

The concordance rate between c-erb-B2 expression of IHC stain on cytoblock and tissue specimen as a gold standard test was 76% compared with the study of Briffod et al. that was 83.6% (13). The positive predictive value of c-erb-B2 expression in IHC on cytoblock specimen in this study was 88%. It means that if the c-erb-B2 expression of IHC stain on cytoblock was positive, the prognosis of tumor is worse (13). The patient could be managed with herceptin more confidently. However, our study shows that negative predictive value was 60%, it means that with negative c-erb-B2 expression of IHC stain on cytoblock, the excisional biopsy will be needed to confirm c-erb-B2 expression of IHC stain on its corresponding tissue samples. The concordance rate between P53 expression of IHC stain on cytoblock and tissue specimen as a gold standard test was 96% compares with the findings of Brifford et al. that was 76.4% (13). The positive predictive value of P53 expression in IHC on cytoblock specimen in this study was 100%. It means the positive P53 expression in IHC on cytoblock makes full confidence to consider bad prognosis of tumor.

The concordance rate between MIB-1 expression of IHC stain on cytoblock and tissue specimen as a gold standard test was 96% as compared to the result obtained by Briffod et al that was 87.0% (13). The positive predictive value of MIB-1 expression in IHC on cytoblock specimen with more

than 10% positive cells was 90.0%. It means the prognosis of tumor should be worse with positive of MIB-1 expression in IHC on cytoblock specimen. According to this study, unfortunately our patients in comparison with references have younger age with higher grade tumors because they have more HER2/neu expression, more Ki67 expression (higher proliferation index) and lower hormonal receptors expression. In our study, concordance of PR and P53 is higher than the other studies, significantly and for the other markers is equivalent or a little higher than the other studies. The positive results of ICC are more reliable for 5 markers, especially for ER and P53 with PPV and specificity equal 100% (table 3).

Because FNA is a simple cost-benefit rapid and non-invasive technique and with it the evaluation of cellular markers by IHC on cell block is possible and results have been acceptable concordance with the usual method (surgery and IHC). Therefore, we recommend to do IHC on cell block from FNA especially for the patients with inoperable cancers or when neoadjuvant treatment is planned.

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