INTRODUCTION
Estrogen and progesterone receptor (ER, PR) levels are important predictors of the breast carcinoma prognosis and play a vital role in the treatment planning. However, some patients with cytosol negative ER and PR, assayed by the quantitative dextran-charcoal assay responded well to a tamoxifen treatment. Clark et al [1] analyzed the correlation between receptor status and patient's characteristics. Over 3000 breast cancer patients had the tumor biopsy specimen examined for the hormonal binding sites. It has been determined that 64% of pre-menopausal and 79% of postmenopausal women had ER-positive tumors. It was also determined that 58% of pre-menopausal and 53% of postmenopausal women had PR positive tumors.

Subsequently, ablation of ovarian functions, resulting in a reduction of estrogen-induced genes and thus suppression of breast cancer growth became a part of therapy in pre-menopausal ER positive patients. The most common approach was to use anti-estrogens, which bind to the estrogen receptor resulting in the inhibition of the estrogen-induced cell proliferation. Tamoxifen, a non-steroid anti-estrogen, given its low toxicity is frequently administered to patients with ER positive pre- and postmenopausal patients. This compound functions by competing with estrogens for binding to the estrogen receptor and therefore interferes with the ability of estrogen-receptor complex to activate transcription. Most recently, tamoxifen was found to trigger apoptosis of breast cancer cells [2]. Several experimental data suggest that binding of tamoxifen to the estrogen receptor is required for this drug to induce cell death [3]. Clinical observations revealed that some patients with cytosol negative ER and PR assayed by the quantitative dextran-charcoal assay (DCA) responded well to the adjuvant therapy with tamoxifen [1]. The aim of the present study was to search for the explanation of this phenomenon.

We have utilized the fluorescent-conjugate cytochemical technique to visualize estrogen and progesterone binding sites in biopsies of the breast cancer at the cellular level. The results were compared to the quantitative cytosol assays for ER and PR in the same biopsies. Additionally, we have analyzed ER and PR in the cytosol of 42 breast cancer biopsies obtained before and after mastectomy to determine whether there is a time-dependent change in the status of these receptors.

RESULTS AND DISCUSSION
Estrogen and progesterone are hydrophobic molecules that are released to the blood stream soon after synthesis. They circulate either free or bound to transport proteins with half-lives ranging from 30 to 90 min. They enter most target cells by diffusion although active transport could occur in some cells. Estrogen and progesterone bind to their receptors upon entering the target cell. Receptors are large protein molecules located in both cytoplasmic and nuclear fractions of the cell. These receptors belong to a large family of structurally related ligand-inducible transcription factors [6].

Recent studies revealed the existence of two different estrogen receptors in humans: ER alpha and ER beta. Although they both bind estrogen as well as other agonists and antagonists, the two receptors have structural differences as well as distinctly different localization and concentration in different organs of human body [6]. Their function is also different: ER alpha and ER beta, when complexed with estrogen, were shown to signal in opposite ways from an AP1 site, with estrogen activating transcription in the presence of ER alpha and inhibiting transcription in the presence of ER beta [8].

The ER ligand, tamoxifen, is an activator of
ER alpha and beta, however the degree of agonism differs between cell types. Tamoxifen acts as an anti-estrogen in breast carcinoma and in the brain while it has beneficial estrogen-like effects in bone and in modulating factors associated with cardiovascular diseases [7].

The ER and PR receptor status helps to predict the response to the treatment of the breast cancer [8]. In patients who are ER and PR positive, the response to the endocrine treatment is about 70% in comparison to less than 10% response of ER and PR negative patients. So, it is important to accurately determine the ER and PR binding sites in the breast cancer biopsies.

The most commonly used assay for ER and PR binding site in the clinical set-up is the dextran-coated charcoal method [8]. This assay requires a significantly large fresh tumor biopsy and cannot identify receptors at the cellular level. It could lead to false negative results due to the presence of a small number of cancer cells positive for ER and PR as well as because of a dilution by the relatively large volume of non-tumor tissue present.

Currently, other quantitative methods have been applied to detect the steroid hormones’ binding sites at the cellular level. These methods include the use of monoclonal antibodies to specific receptors. Advantages over the dextran-coated charcoal assay include no need for the radiolabeled compounds, faster time in obtaining results and much smaller sample of tissue required. This technique does not need the presence of the functional receptor, which is required by the dextran-coated charcoal assay, to be detected [8].

Commercial kits are available and those assays are used in clinical settings. However, all those assays require a tissue homogenate, so the cellular localization of hormonal binding sites in cancer cells is not possible.

In contrast, immunocytochemical methods allow direct visualization of hormonal binding sites in the specimen and correlation of morphology with receptor status. The avidin-biotin-peroxidase method is frequently used because it allows the study of receptor status in paraffin-embedded tissue as well as in frozen sections and visualizes the receptor in the tumor cells. The disadvantage of this technique is that it is semi-quantitative. We have elected to use the cytochemical fluorescence assay in this study.

Results obtained in the present study revealed that about 70% of tested tumor biopsies were positive for estrogen binding sites and about 60% were PR positive using the quantitative technique. This is consistent with findings by [1]. These biopsies also contained tumor cells positive for ER and PR as assayed by qualitative fluorescence methods. However, about 25% of biopsies, which were negative by a quantitative assay had tumor cells positive for ER and PR as assessed by qualitative fluorescence methods.

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Results obtained in the present study revealed that about 70% of tested tumor biopsies were positive for estrogen binding sites and about 60% were PR positive using the quantitative technique. This is consistent with findings by [1]. These biopsies also contained tumor cells positive for ER and PR as assayed by qualitative fluorescence methods. However, about 25% of biopsies, which were negative by a quantitative assay had tumor cells positive for ER and 15% were positive for PR by the fluorescence study. This alone could account for the beneficial clinical response to anti-estrogen therapy in the ER-negative breast cancer patients reported in the literature. These differences could be attributed to many factors including the number of cancer cells in the biopsy, the heterogeneity of cell population, or detection of type II or III estrogen binding sites by the cytochemical fluorescence assay.

To address this problem in the clinical settings, new techniques visualizing hormonal binding sites within the cancer cells are desirable to improve the management of patients with hormone-dependent malignant diseases.

REFERENCES

Figure 1: Breast biopsy, estrogen receptor (by fluorescence) positive, cytosol positive; 66 fmol. Bar = 50 µm.

Figure 2: Breast biopsy, progesterone receptor (by fluorescence) positive, cytosol positive, 175 fmol. Bar = 50 µm.

Figure 3: Breast biopsy, estrogen receptor (by fluorescence) positive, cytosol negative; 0 fmol. Bar = 50 µm.

Figure 4: Breast biopsy, progesterone receptor (by fluorescence) positive, cytosol negative; 0 fmol. Bar = 50 µm.