SUMMARY

Human breast is generally considered to be a passive organ being a target tissue for both steroids and peptide hormone. However, in an article it was suggested that breast may also be a primary endocrine organ. Prolactin is known to affect the milk secretion but whether other peptide hormones have any direct action on breast is unknown. However leutinizing hormone releasing hormone analogue has been shown to be effective in treatment of advanced breast cancer.

Steroid hormones with estrogenic properties are largely accepted as potential promoters in the progression of human breast cancer and act through hormone-specific receptor protein.

It has been recognised for a number of years that pituitary prolactin has also a powerful stimulating effect on the breast and on some types of breast cancer in experimental animals.

If prolactin promotes human breast cancer, then it would be expected that both the concentration of this hormone in the blood as well as the prolactin receptor concentration would influence prognosis in patients. Studies on prolactin levels in patients are controversial, may be because of number of factors which influence its secretion.

Several mammary carcinoma cell lines were found to have prolactin binding sites and prolactin was found to facilitate their growth. Specific binding of human prolactin of greater than 1% was seen in good percentage
human breast tumours. Therefore in view of all these observations it would be interesting to see if there are any functional correlation and changes in the pathological state (benign breast disease and breast cancer) of binding with the antibodies. Such a finding will open gates for a large number of experimentation and treatment for breast disease.

Keeping this in view the present study was undertaken to see if there are any specific binding sites for the peptide hormones in normal and pathological breast tissue. This was ascertained immunocytochemically. An attempt was also made specially in case of prolactin to biochemically quantitate these receptors in crude as well as in pure plasma membrane preparations.

The first part of this study was immunocytochemical localisation of peptide hormone (PRL, FSH, LH) binding sites in benign and malignant human tissue. This was accomplished by using a highly sensitive immunocytochemical method, the dinitrophenyl hapten sandwich staining procedure and polyclonal antisera against peptide hormones. Both prospective and retrospective sections of formalin fixed paraffin embedded tissue of breast disease patients were immunostained. In this part of study an initial attempt was also made to immunostained fine needle aspirates of breast disease patient for prolactin binding sites.

One hundred and seven formalin fixed paraffin embedded breast tissue sections were immunostained for localisation of prolactin binding sites in benign and malignant breast lesions. Among these thirty five and twenty eight sections were also stained for FSH and LH binding sites respectively. An
appropriate control slides were included in each batch of staining. Eighty two percent of breast carcinomas gave an intracellular positive reaction in tissue section for prolactin binding sites whereas, it was seventy two percent in fine needle aspiration. The intracellular localisation of prolactin could be the result of hormone internalisation. In both the cases heterogeneity of binding was shown. In some cases irregular staining of the cell periphery was also observed.

A definite relationship between age of the patients and intensity and occurrence of positive staining for prolactin was observed. Majority of the breast carcinoma slides showed positive reaction in tumours removed from patients during the peri and post menopausal periods. Whereas patients before 40 years of age showed poor or irregular staining. Also poorly differentiated tumours gave less intense staining though metastasing tumours showed stronger reactions.

Amongst the benign lesions immunostained, majority were fibroadenoma of which 54% of cases showed positive staining for prolactin. The cells showing apocrine metaplasia associated with fibroadenoma were more intensely stained as compared to the ductular lining epithelial cells of fibroadenoma. Lobular hyperplasia, lactating adenoma and galactocele were also positively stained whereas epitheliosis and papillomaloid hyperplasia were heterogeneously stained.

Staining for FSH and LH binding sites in breast tissue sections was only a preliminary study. Though weak staining was obtained in most cases of carcinomen slides studied, no conclusion could be made for this binding.
The results of immunostaining on FNAC smears were comparable with that of histologic sections. Sufficient cells could be obtained by this method that can be immunostained for determining binding sites for prolactin. Fine needle aspiration cytology being economical, quick and non-traumatic procedure could be more readily used for the hormonal study.

In the second part of this study an attempt was made to biochemically quantitate prolactin receptors. For this both purified and crude plasma membranes of benign and malignant tissues were prepared.

Pure plasma membrane were prepared by Sucrose density gradient method. The purity of the plasma membrane was checked by enzyme assay, electron microscope and electrophoresis. Western blotting of two pure plasma membrane preparations of malignant tissue were done and immunostained with DNP localisation immunochemical method. Both pure plasma membrane preparations and crude membranes were used for binding assays. Radioactive labelling of prolactin with radioactive iodine was done using Chloramine T as oxidising agent and the radioactive hormone was purified by column chromatography using Sephadex G-75. Three peaks of radioactivity were eluted, the first represented the damaged hormone aggregates, the second represented the labelled hormone and third was of free iodine. The specific activity of purified labelled hormone was calculated.

Before the binding assay of prolactin binding sites, desaturation of occupied receptors from endogenous prolactin was done since prolactin does not appear to dissociate from its receptors during membrane preparation. For this, membranes were treated with high concentrations of MgCl₂ prior to binding assays.
For the binding assay, membrane preparations were incubated with radio active hormone (PRL) overnight in the absence and presence of an excess of cold hormone. Specific binding was calculated as the difference between the radio activity bound in the absence (total binding) and in the presence of an excess cold prolactin (non-specified binding). These data were expressed as the percentage of the total radioactivity added.

Pure plasma membrane preparation of malignant tissue that were checked by electron microscope and by the presence of positive marker enzyme showed two bands by immunobloting. These bands may be considered to be of prolactin (25 K Dal) and its variant (29 K Dal). Binding study for prolactin had shown low amount of prolactin receptor in crude malignant breast tissue. However the purified plasma membrane of these did not show any binding for prolactin.

From the present study it could be concluded that there are specific binding sites for peptide hormones in human pathological breast tissue. These could be located immunocytochemically in the formalin fixed paraffin embedded breast tissue slides as well as in fine needle aspirates of the breast lumps. The staining was heterogenously distributed. Breast carcinoma showed more consistent staining as compared to benign breast disease. Also prolactin receptors could be located biochemically in the malignant breast tissue membranes preparations. Prolactin binding sites were present in greater proportion in peri or post menopausal breast cancer patients. Prolactin receptor concentration in post menopausal patients were consistent with the results of the study that the prolactin plasma levels are elevated in post
menopausal patients. Post menopausal patients were also reported to have more estrogen receptors. Estrogens are reported to stimulate prolactin secretion and increase prolactin receptors in breast. Presence of prolactin binding sites in metastasing tumours may also indicate that aggressive breast cancer were associated with the secretion of an unknown factor acting at the hypothalamic pituitary level to enhanced prolactin secretion which in turn increases tissue prolactin binding sites, or perhaps that the tumour itself secretes prolactin.

The presence of prolactin binding sites in malignant tumours of breast would thus indicate that prolactin might be significant in the support of breast cancer growth in vivo.

Presence of prolactin binding sites in benign breast disease is of great importance. The apocrine change that is associated with high breast cancer risk had specific prolactin binding sites. This finding may also suggest a role of prolactin in the etiology of breast cancer through the indication of apocrine change.