Estrogen receptors in human thyroid gland

An immunohistochemical study

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ABSTRACT

Objective: Thyroid diseases affect women approximately 3 times more frequently than men. It has been suggested that the female sex steroids stimulate thyroid growth such as in the breast. Seventeen beta-estradiol, the major estrogen in the body acts via estrogen receptors (ER) present in the nucleus of the cell. The aim of the study is to determine the ER status in the thyroid gland tissues.

Methods: Our study was based on immunohistochemical staining for ER. Fifty previously diagnosed cases of various thyroid lesions were selected from the Surgical Pathology Records of Pathology Department, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi, Pakistan between March and August 2000. The staining was performed on formalin-fixed paraffin embedded tissues using monoclonal anti-ER antibody (clone 1D5). Out of 50 cases, 8 were nodular goiter, 9 cases of adenoma, 19 papillary carcinoma, 10 follicular and 4 cases were of medullary carcinoma. Surrounding normal tissue was available in 25 (50%) cases, 4 non-neoplastic and 21 neoplastic lesions. Out of 50 cases, 10 (20%) were males and 40 (80%) were females, the youngest patient was a 14-year-old female and the eldest patient was a 56-year-old male.

Results: Despite the availability of normal thyroid tissue and a wide range of lesions, none of our cases showed positive staining.

Conclusion: In contrary to many earlier reports by immunohistochemical method using monoclonal antibody (clone 1D5) on formalin-fixed paraffin-embedded thyroid tissues, the ER are not detectable. The effect of estrogen on thyroid gland may be indirect one.

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Epidemiological evidence indicates that the incidence of thyroid disorders is greater in women than in men. Thyroid cancer is 2.5 times more common in females than in males during reproductive life. It is nearly equal to one in children, increases abruptly to approximately 3 at puberty and remains at this level until menopause. Then it begins to decline, reaching 1.5 by the age of 65 years.[1] A history of one or more pregnancies, use of lactation suppressants, oral contraceptives, increased body weight and irregular menstruation are all associated with an increased risk of thyroid cancer,[2,3] suggesting a role for sex steroids. Seventeen beta-estradiol is primary estrogen of ovarian origin. It is also produced in the placenta and by the

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peripheral aromatization of androgens. It acts via a ligand-activated transcription factor, estrogen receptor (ER), located in the nucleus of the cells.[4,5] Estrogen receptor has been demonstrated not only in various normal and neoplastic reproductive organs but also in organs such as breast, kidney, colon and brain.[6,7] It is well established that there is a sound correlation between ER status in breast carcinoma and patients' survival and response to endocrine therapy.[8,9] The purpose of the present study was to ascertain the ER status in normal and abnormal thyroid tissue, because the demonstration of ER in the nuclei of thyroid cells is essential if one is to implicate estrogenic effect in biological evolution of thyroid lesions.[10]

Methods. Fifty previously diagnosed cases of various thyroid lesions were selected from the Surgical Pathology Records of the Pathology Department, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi, Pakistan between March and August 2000. For immunohistochemical staining, the sections were mounted on poly-L-lysine (Sigma Diagnostics) coated slides and allowed to dry in an oven at 56-60°C for one hour. The antigen retrieval was achieved by 2 procedures. Thirty slides were treated in the water bath and 20 slides in microwave oven. The slides were immersed in 10mM citrate buffer at pH 6.0 in both procedures. In the water bath, the slides were heated at 95-99°C continuously for 40 minutes. In the microwave oven, the sections were treated twice, each for a 10 minute cycle, the power was adjusted to make sure that buffer boils for at least 7 minutes in each cycle. Monoclonal mouse anti-ER antibody clone 1D5 was procured from Dako Corporation Denmark and used as primary antibody. It reacts with the N-terminal domain of ER,[11] and strongly labels the nucleus of the cells known to contain abundant amounts of ER. In breast carcinoma, the ER status determined by this new antibody clone (ER1D5), using antigen retrieval techniques, in routine formalin-fixed paraffin embedded sections gives significant correlation with endocrine response.[12-14] Universal Dako LSAB-2 Kit, ALP (code No. K676) was procured from Dako Corporation Denmark and used as visualizing system. It utilizes a refined avidin-biotin technique in which a biotinylated secondary antibody reacts with several alkaline phosphatase-conjugated streptavidine molecules. With the addition of substrate-chromogen solution, a brown color is produced at the site of reaction. All the reagents were used according to manufacturers' instructions. Ten unstained formalin-fixed paraffin embedded control tissue sections mounted on silinized slides were procured from Dako (code No. T1074). A slide (positive control) was run with each batch to assure reliability of reagents and procedure. To assure the reliability of processing in the department, endometrial tissue sections (another positive control) from the department were mounted on poly-L-lysine coated slides and were run with each batch. For negative control slides instead of using the primary antibody, fetal calf serum was used, which was provided with the primary antibody. There is no general agreement as to how the immunohistochemical assay should be evaluated and several different methods of scoring have been described.[15] In the present study slides were labeled as ER positive if any degree of positive staining was observed in any number of cells.

Results. In the present study, of the 50 cases selected, 8 were nodular goiter, 9 cases of adenoma, 19 papillary carcinoma, 10 follicular carcinoma and 4 cases were of medullary carcinoma. Surrounding normal tissue was available in 4 non-neoplastic and 21 neoplastic lesions. In all the lesions, the cases were selected representing both sexes (**Table 1**). Out of 8 cases of nodular goiter 2 belonged to males and 6 were from female patients. Of 42 neoplastic cases 8 belonged to males and 34 to females. Overall male patients comprised 20% (10 cases) and females 80% (40 cases). Cases were selected representing wide age ranges (**Table 1**). The youngest patient was a 14-year-old female with papillary carcinoma and the oldest patient was a 56-year-old male again with papillary carcinoma. Despite the consistently positive staining in control slides (**Figure 1 & 2**), wide range of available tissues, in different age groups in both sexes, none of our cases including surrounding normal thyroid tissue showed positive staining for ER.

Discussion. It is generally accepted that for a steroid hormone to have a direct effect on target organs, presence of a specific, high affinity intracellular receptor is an absolute prerequisite.[16] This concept is further substantiated by the findings that abundant amount of ER is present in target tissues. Furthermore, at least in breast tumor ER status affects clinical behavior, prognosis and response to hormonal therapy.[17] In thyroid, this relationship is exemplified by the presence of thyroid stimulating hormone receptors and their therapeutic importance in thyroid neoplasm.[18] In fact, detection of ER in thyroid has been the object of various

Table 1 - Distribution of cases according to sex and age.

Diagnosis	Male N = 10	Female N = 40	Age range (years)
Nodular goiter	2	6	22 - 50
Adenoma	2	7	27 - 45
Papillary CA	2	17	14 - 56
Follicular CA	2	8	23 - 45
Medullary CA	2	2	33 - 40

CA - carcinoma

Table 2 - The comparison of various studies of estrogen receptors in thyroid tissues.

Study	Year	n of cases	Assay method	Cut-off value	ER+ cases
Molteni et al[7]	1981	7	SDG	3 fmol/mg	2/7
Diaz et al[10]	1991	80	IHA		32/80
Chaudhury et al[16]	1986	45	SDG	1 fmol/mg	23/45
Clark et al[19]	1985	15	SDG	0.2fmol/mg	14/15
Marugo et al[20]	1989	30	DCC	3 fmol/mg	37/60
Miki et al[21]	1990	88	DCC	1 fmol/mg	20/88
Metaye et al[22]	1993	42	EIA		29/42
Hoeven et al[23]	1993	135	DCC	1 fmol/mg	61/135
Bonacci et al[24]	1996	48	EIA	1 fmol/mg	18/48
Frolich et al[30]	1990	23	IHA		0/23
Jaklic et al[31]	1995	11	IHA		0/11
Giani et al[32]	1993	34	ICA		0/34
Present study	2002	50	IHA		0/50

SDG - sucrose density gradient,
DCC - dextran-coated charcoal,
IHA - immuno-histochemical assay,
ICA - immuno-cytochecmical assay,
EIA - enzyme-immuno assay, ER - estrogen receptor

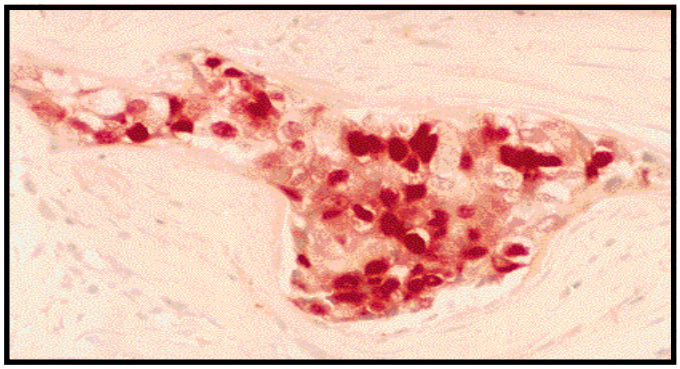

Figure 1 - Breast carcinoma (positive control slide provided with the kit) showing estrogen receptor positivity confined to the nuclei of the tumors cells. (Immunostain x 2000)

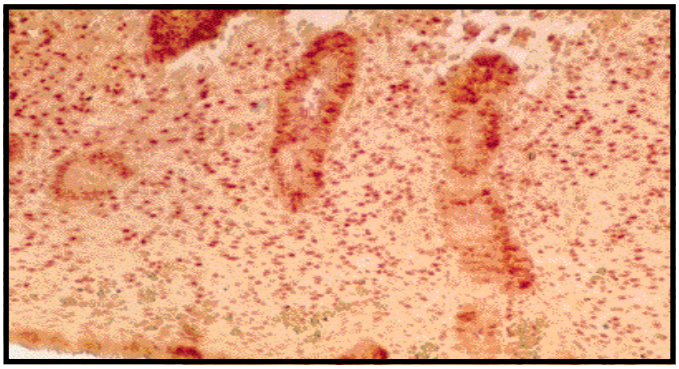

Figure 2 - Endometrial tissue (internal positive control) showing nuclei of the epithelial and stromal cells positively stained for estrogen receptors. (Immunostain x 1000)

studies. **Table 2** summarizes the different studies, which have been carried out for the determination of ER. In most studies chemical methods (dextran-coated charcoal, sucrose density gradient, and enzyme immunoassay) have been employed detecting cytosolic fraction. These studies indicate a high prevalence of thyroid specimens positive for ER, however, the concentration is generally very low and the distribution is ubiquitous in normal and pathological thyroid tissues.[7,16,20-24] No clear relationship was found with the clinical or pathological features or, in case of carcinoma, to subsequent metastatic potential.[23] Our results are not in agreement with these findings. Discrepancies in results could stem from several sources, including source of tissue, threshold set for positivity and different antigenicity of ER in thyroid gland. In chemical assays cytosolic extract prepared from fresh tissue homogenates is utilized and the result is expressed in femto-moles per milligram of cytosole. Using immunohistochemical procedures, in the breast, it has been shown that ER proteins are exclusively nuclear proteins and that the cytosolic fraction is likely to be an artifact of homogenization.[25,26] Unlike breast, in most of the studies in thyroid tissues the cut-off limits for positivity are 1-3 fmol/mg, which is very low. It has been found in the breast that a level of 20 or more fmol/mg corresponds well with the response to hormonal therapy.[27,28] It is possible that with IHA such low levels are not detectable. Chemical methods detect only free ER because the reaction site used for ER detection and the binding site of endogenous hormone are same. We have used mouse anti-human monoclonal antibody, clone 1D5, used effectively on clinical basis to detect ER in breast tissue.[29] It reacts with N-terminal domain (A or B region) of human ER, an epitope different from the binding site of endogenous hormone on the receptor molecule. It is possible that some non-classical ER, with N-terminal domain different from breast, is present in thyroid. In fact, a 2nd subtype of ER (ER beta) has been detected recently and has forced endocrinologists to re-evaluate many aspects of estrogen physiology.[5] Results of the present study are consistent with Frolich et al,[30] and Jaklic et al.[31] Using immunohistochemical staining both concluded that ER proteins are neither significantly detectable nor pertinent for follow-up in the patients with thyroid neoplasia. Although, Giani et al,[32] demonstrated positive ER staining in 24 out of 34 cases

but concluded that to be an artifact, activity of endogenous peroxidase on chromogen solution. We excluded peroxidase-antiperoxidase complex from the visualizing system, used alkaline phosphatase and found no staining. Endogenous alkaline phosphatase activity is destroyed in paraffin sections during processing.[33] Since it was a retrospective study, a limitation of our study was our lack of control in tissue handling at the time of processing. This could result in the loss of antigenicity. To deal with this potential problem, with each batch, we stained sections from endometrial blocks processed in the same department as control to assess antigen preservation and antigen retrieval. We were able to detect positive staining in almost all sections, and, therefore, we believe that our lack of ER detection is not a result of antigen degradation during processing. Although our results agree with those of some studies and are different than the others, there is not strong support from any of the studies that thyroid tissues have a significant degree of ER positivity. Various epidemiological and experimental observations, as described earlier, point to the role of sex steroid hormones in the causation of thyroid diseases and their prognosis. The nature of this mechanism is not known, but according to our study it seems unlikely to be a direct one. In a recent study,[34] 76% of the central histaminergic neurons of tubero-mammillary complex of the caudal diencephalon were found to be ER positive. Luteinizing hormone-releasing hormone (LHRH) neurons are ER negative. Authors concluded that the estrogenic effect in the induction of the preovulatory LH surge is mediated by these ER positive neurons that relay the steroid signals to LHRH neurons. In synchrony with these findings it should be investigated whether the effect of estrogen on thyroid gland is mediated through some central mechanisms, either in the pituitary gland or in hypothalamus.

In conclusion, in contrary to many earlier indications, by immunohistochemical method using monoclonal antibody (clone 1D5), on formalin-fixed paraffin embedded tissues, the ER are not detectable in normal or pathological thyroid tissue. The effect of estrogen on thyroid gland may not be a direct one.

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