Original Article

Immunohistochemical Study of Progesterone Receptors in Thyroid Gland

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Abstract

Objective: To determine the progesterone receptor status in thyroid gland.

Methods: This study was based on immunohistochemical staining of formalin fixed paraffin embedded tissues, for progesterone receptors, in 50 previously diagnosed cases of various thyroid lesions and surrounding normal thyroid tissue.

Results: Out of 50 cases, 8 were nodular goiter, 9 cases of adenoma, 19 papillary carcinoma, 10 follicular carcinoma and four cases were of medullary carcinoma. Surrounding normal tissue was available in 4 non-neoplastic and 21 neoplastic lesions. Overall male patients comprised 20% (10 cases) and females 80% (40 cases). Although a wide range of lesions in both the sexes including wide age range were available, none of our cases were positively stained for progesterone receptors.

Conclusion: In contrary to earlier reports by immunohistochemical method using monoclonal mouse anti-PR antibody clone PgR 636, on formalin-fixed paraffin embedded thyroid tissues, the progesterone receptors were not detectable in our human samples. The effect of progesterone on thyroid gland may be an indirect one (JPMA 55:321;2005).

Introduction

The incidence of thyroid disorders is greater in women than men. Thyroid cancer is approximately 2.5 times more common in females; however, under the age of 10 years, the incidence is nearly equal in both genders. This near equality changes abruptly around puberty so that the ratio of females to males is 3:1 in the 10-19 year age group. This ratio declines steadily near menopause in women reaching to 1.5 by 65 years of age.[1] A history of one or more pregnancies, use of drugs suppressing lactation, oral contraceptives, increased body weight and irregular menstruation are associated with an increased risk of thyroid cancer[2], suggesting a role of sex steroid hormones.

Progesterone is secreted by the corpus luteum during the luteal phase of menstrual cycle. The ovarian progesterone significantly contributes to cellular proliferation and function of female reproductive tissues through a hormonally regulated intracellular transcription factor - progesterone receptor (PR). The presence of sex steroid receptors was first demonstrated in 1960. Since then steroid receptor analysis has occupied a critical role in evaluation of patients with carcinomas.[3] In breast carcinoma, an established correlation exists between the PR status, patients' survival and response to endocrine therapy. Therefore it is of value in planning therapy and predicting disease course in breast cancer patients.[4] The aim of this study was to demonstrate the PR status in diseased and surrounding normal thyroid tissue, because their presence is essential if one is to implicate progestinic effect in biological evolution and therapy of thyroid lesions.

Methods

Fifty diagnosed cases of various thyroid lesions were selected from the records of the Pathology Department, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi, Pakistan, from March to August 2000. Fresh sections from paraffin embedded blocks were stained and reviewed. For immunohistochemical staining, the sections were mounted on poly-L-lysine (Sigma Diagnostics) coated slides and allowed to dry in the oven at 56-60^{0}C for one hour. The slides were immersed in 10mM citrate buffer at pH 6.0. The antigen retrieval was achieved by two procedures: 30 slides were treated in water bath and 20 slides in microwave oven. In water bath, the slides were heated at 95-99^{0}C 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-PR antibody clone PgR 636 was procured from Dako Corporation Denmark and used as primary antibody. Anti-PR antibody reacts with the human progesterone in the nucleus of cells expressing the receptor.[5] This antibody is used for the immunohistochemical detection of progesterone receptors in normal and neoplastic tissues for prediction of tumor response to endocrine therapy and patient outcome.[6] 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 the manufacturers' instructions. Ten unstained formalin-fixed paraffin embedded control tissue sections mounted on silinized slides were procured from Dako (Code No. T1382). 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 was 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 provided with the primary antibody was used. There is no general agreement as to how the immunohistochemical assay should be evaluated and several different methods of scoring have been described.[7] In the present study slides were labeled as PR positive if any degree of positive staining was observed in any number of cells.

Results

Of the fifty cases selected, 8 were nodular goiter, 9 adenoma, 19 papillary carcinoma, 10 follicular carcinoma and four cases were of medullary carcinoma. Surrounding normal tissue was available in 4 non-neoplastic and 21 neoplastic lesions. Table 1 shows age and sex distribution in different thyroid lesions selected.

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

Figure. Positive control slide provided with the kit (breast carcinoma), showing positivity for progesterone receptors confined to the nuclei of the tumor cells (Immunostain 20x).

Overall male patients comprised 20% (10 cases) and females 80% (40 cases). The youngest patient was a 14-year-old female and the oldest patient a 56-year-old male, both with papillary carcinoma. Despite the consistently positive staining in control slides (Figure 1), wide range of available tissues, in different age groups in both sexes, no positive staining for PR was observed in our selected sample including surrounding normal tissues.

Discussion

It is generally accepted that for a steroid hormone to have a direct effect on target organs, presence of specific, high affinity intracellular receptor is an absolute prerequisite.[8] This concept is further substantiated by the findings that abundant amount of PR is present in target tissues. Furthermore, at least in breast tumour, PR status affects clinical behaviour, prognosis and response to hormonal therapy.[9] In thyroid this relationship is exemplified by the presence of thyroid stimulating hormone receptors and their therapeutic importance in thyroid neoplasms.[10] The objective of various studies had been the detection of PR in thy-

Table 2. Comparison of various studies of progesterone receptors in thyroid tissue.

Study	Year	No. of cases	Assay method	Cut-off Value	PR + cases
Chaudhury et al[8]	1986	25	SDG	1 fmol/mg	7/25
Marugo et al[13]	1989	30	DCC	3 fmol/mg	29/30
Money et al[17]	1989	22	ICA		4/22
Miki et al[11]	1990	43	DCC	1 fmol/mg	7/43
Hoeven et al[14]	1993	135	DCC	10 fmol/mg	68/135
Giani et al[22]	1993	34	ICA		0/34
Jaklic et al[23]	1995	11	IHA		0/11
Bonacci et al[12]	1996	48	EIA	1.5	22/48
Present study	2004	50	IHA	fmol/mg	0/50

SDG: Sucrose density gradient; DCC: Dextran-coated charcoal; ICA: Immuno-cytochemical assay; IHA: Immuno-histochemical assay; EIA: Enzyme-immuno assay; PR: Progesterone receptor.

detection of PR in thyroid as summarized in Table 2. In majority of studies chemical methods (dextran-coated charcoal, sucrose density gradient and enzyme-immuno assay) have been employed detecting cytosolic fraction. These studies indicate the presence of PR about half of the specimens, however the concentration is very low and the distribution is ubiquitous in normal and diseased thyroid tissues.[8,11,12] Although higher content of PR have been reported by Murago et al[13], and Hoeven et al[14], but no clear relationship was found with the clinical and pathological features. Our results are not in agreement with the above results. Discrepancies in result could stem from several sources, including source of tissue, threshold set for positivity, accuracy of the method and different antigenicity of PR in thyroid gland. In chemical assays, cytosolic extract is prepared and PR is detected in cytosole. It has been shown that sex-steroid receptor proteins are exclusively nuclear proteins using immunohistochemical procedures in breast[15,16] and thyroid.[17] The cytosolic fraction is likely to be artifact of homogenization. Unlike breast, in most of the studies the cut-off limits for positivity are low i.e. 1-3 fmol/mg in thyroid tissues. In breast it has been found that a level of $\geq$ 10 fmol/mg is significantly prognostic.[15] It is likely that IHA are unable to detect such low levels. It has been shown by DCC that 70% of meningiomas contain a large quantity of PR, positive ICA for PR in the nuclei was seen in only in 10% of the tumors.[18] The lack of clinical response to medroxyprogesterone acetate[18], further supported the contention that PR proteins detected by DCC are different form the proteins detected by ICA and are not functional. Like PR, estrogen receptor proteins were also detected in various nontarget organs including thyroid, by chemical methods in different studies. Recent studies using IHA on thyroid[19] and colon[20] have not confirmed these earlier reports and no estrogen receptor has been detected. In fact it has been suggested that the presence of DCC-detectable steroid receptors should be verified by the ICA and respective mRNA expression.[21] Results of the present study are consistent with Giani et al[22], and Jaklic et al.[23] Using IHA, both concluded that PR proteins are neither significantly detectable nor pertinent in patients with thyroid neoplasia. Although Giani et al, demonstrated positive PR staining in 22 out of 34 cases but concluded that to be an artifact activity of endogenous thyroperoxidase on chromogen solution. We excluded peroxidase- antiperoxidase complex from the visualizing system, used alkaline phosphatase and found no nonspecific background staining. Endogenous alkaline phosphatase activity is destroyed in paraffin sections during processing.[24]

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 PR 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 no strong support from any of the studies that thyroid tissue has a significant degree of PR positivity. Various epidemiological and experimental observations point to the role of sex steroid hormones in the causation and prognosis of thyroid diseases. The nature of this mechanism is unclear, but according to the results of our study it seems unlikely to be direct. In a recent study[25], 76% of the central histaminergic neurons of tubero-mamillary complex of the caudal diencephalons were estrogen receptor (ER) positive. Leutinizing hormone-releasing hormone neurons are ER negative. Authors of the study concluded that estrogenic effect in the induction of preovulatory LH surge is mediated by these ER positive neurons. In synchrony with these findings, it should be investigated whether the effect of progesterone on thyroid gland is mediated through some central mechanisms, either in pituitary gland or in hypothalamus.

In conclusion, and contrary to many earlier studies, by immunohistochemical method using monoclonal antibody (clone PgR 636), on formalin-fixed paraffin embedded tissues, the PR are not detectable in pathological and surrounding normal thyroid tissue. The effect of progesterone on thyroid gland seems unlikely to be direct.

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