# Immunohistochemical study of oestrogen receptors in 351 human thyroid glands

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## ABSTRACT

**Introduction: It is well recognised that the pathogenesis of thyroid diseases is complex and different factors such as genetic factors, iodine deficiency, sex, age, radiation therapy in childhood, growth stimulating antibodies, and other epithelial growth factors can influence them. Epidemiological features of thyroid tumours and experimental evidence suggest that female sex hormones may exert effects on the thyroid gland and its neoplasms. This possibility was addressed by investigating the expression of oestrogen receptor protein in 351 thyroid lesions.**

**Methods: The tissues from 351 human thyroid glands comprising 130 nodular goitres and 221 neoplastic lesions were used for the present immunohistochemical assessment of oestrogen receptor expression.**

**Results: Incidence of oestrogen receptor positive cases were 24 percent (31/130) for nodular goitres, 22 percent (8/37) for follicular adenomas, 11 percent (2/18) for follicular carcinomas, 31 percent (37/119) for papillary carcinomas, zero percent (0/35) for medullary carcinomas and zero percent (0/12) for undifferentiated carcinomas. The incidence of oestrogen receptor positivity, which is compatible with other studies, is higher in well-differentiated thyroid lesions. The incidence of oestrogen receptor reactivity does not significantly differ between females and males of different age groups and it does not correlate with lymph node status, and vascular and capsular invasions.**

**Conclusion: The relatively high proportion of oestrogen receptor positivity in goitres, follicular adenomas and papillary carcinomas, compared with its reactivity in other thyroid neoplasms, and contrasted against normal thyroid tissue, suggests that the incidence of oestrogen receptor reactivity tends to increase with better differentiation of thyroid lesions. This finding may have clinical relevance.**


**Keywords: follicular adenoma, goitre, immunohistochemistry, oestrogen receptor, papillary carcinoma, thyroid cancer**

*Singapore Med J 2007; 48(8):744–747*

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## INTRODUCTION

The pathogenesis of thyroid cancers is complex, and factors such as iodine deficiency, genetic factors, gender, age, the history of irradiation in childhood, thyroid growth stimulating antibodies and epithelial growth factors, can possibly affect its development. Moreover, benign and malignant thyroid lesions are more common in females, and the prognosis is also more favourable. This difference may be due to the effect of sex hormones.[1-4] Follicular adenomas and well-differentiated carcinomas occur predominantly in women and are most frequent in postpubertal and premenopausal age groups.[3-8] These epidemiological associations have led to recent investigations that confirm the presence of oestrogen and progesterone receptors in both neoplastic and nonneoplastic human thyroid tissues.[4,8-14] This has prompted research into the presence of sex hormone receptors in thyroid diseases.

Thyroid lesions in many women occur during the childbearing age, when the hormonal levels are at their maximum concentration. The prevalence of thyroid carcinoma is three times more in women of childbearing age than in men. Women during premenarche and after menopause have a lower prevalence. This difference is observed irrespective of geographic location. The increased risk is also seen in women taking oral contraceptive pills. Proving the presence of the oestrogen receptor (ER) in the nuclei of thyroid lesion cells is mandatory for their biological evaluation. Not only will their dependence on oestrogen for development or progression be implied, but it may also indicate that antioestrogenic drugs may help stop growth and proliferation of the neoplastic cells. This theory is already proven in breast and prostate cancers where the use of antihormonal drugs has shown significant improvement in prognosis.[5]

Previous researchers examining this issue included only a relatively small number of patients. Moreover, few researchers have examined the correlation between receptor content and clinical behaviour of the thyroid lesions in detail. The significance of these receptors is still unclear.

In the study by Kishino et al, the antitumour effect of tamoxifen in high doses for multidrug-resistant anaplastic thyroid carcinoma was considered beneficial.[15] Other studies in this particular field include: Hiasa et al examined 313 paraffin blocks of thyroid lesions with immunohistochemical staining for the presence of ERs,[16] Takeichi et al, in a similar study, compared the number of ER positive cells in different thyroid lesions,[17] and Yane et al assessed the presence of ER in frozen-section specimens.[18] In this study, we evaluated ER reactivity using immunohistochemical methods in both neoplastic and nonneoplastic thyroid lesions, and its association with demographical differences in patients. We used the avidin-biotin-peroxidase method within formalin fixed, paraffin embedded blocks to study the thyroid lesions.

## METHODS

We searched the files of the pathology department of Shariati Hospital, a referral hospital affiliated to Tehran University of Medical Sciences, and selected patients who underwent thyroidectomy from 1999 to 2004. There were 404 consecutive thyroidectomy specimens and in 351 of them, paraffin blocks and necessary clinical data were available. Immunohistochemical studies were done on sections from paraffin blocks. The records of patients were obtained from the medical records department of the hospital and also from the pathology reports. The age, gender, type of lesion, presence of vascular, capsular and lymph node invasions were recorded.

351 previously-diagnosed cases of different thyroid lesions were selected. Specimens comprised 351 paraffin blocks of thyroid lesions, including 130 (37%) goitres, 119 (34%) papillary carcinomas, 35 (10%) medullary carcinomas, 12 (3%) undifferentiated carcinomas, 18 (5%) follicular carcinomas (all widely invasive) and 37 (11%) follicular adenomas. Overall, we had 130 goitres (37%) and 221 (63%) neoplastic lesions. The selected paraffin blocks had minimal haemorrhage and necrosis, good quality fixation and some normal thyroid tissue. Malignant lesions were also reviewed for vascular or capsular invasion, and lymph node involvement. The cases were divided in three age groups: premenopausal women, postmenopausal women, and men of different ages.

Prior to staining, the slides were rehydrated in serial dilutions of ethanol and deparaffinised in xylenes.

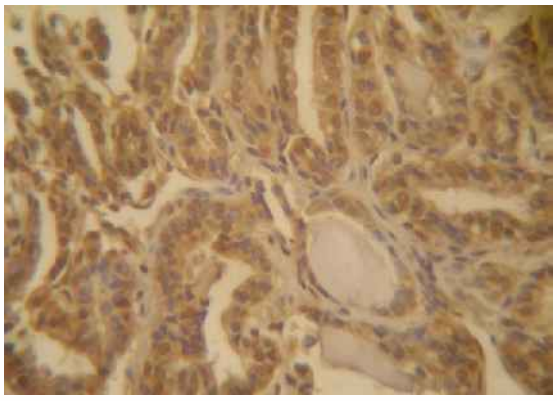

**Fig. 1** Immunohistochemical staining with ER antibody shows positive ER reactivity in nuclei in papillary carcinoma (× 400).

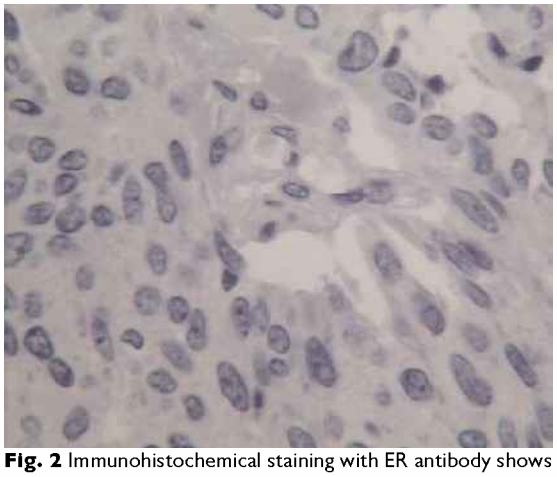

**Fig. 2** Immunohistochemical staining with ER antibody shows negative ER reactivity in medullary carcinoma (× 400).

Sections were mounted on poly-L-lysine-coated slides and allowed to dry in an oven at 56–60°C. The antigen retrieval was achieved in a microwave oven. Monoclonal mouse anti-ER antibody clone 1D5 was procured from Dako Corporation, Denmark and used as the primary antibody. We utilised the avidin-biotin-peroxidase technique. Upon adding the substrate-chromogen solution, a brown colour was produced at the site of reaction. All the reagents were used according to instructions given by the manufacturers. A positive control slide prepared from breast carcinoma known to be positive for ER was run with each batch to assure the reliability of reagents and the procedure. Negative control slides were prepared from the same tissue block, but incubated with PBS, instead of the primary antibody. Lesions were interpreted as positive if at least 10% of tissue showed reactivity.[5] Immunoreactivity in all the lesions occurred in the nuclei as expected. Data was analysed with the Statistical Package for Social Sciences version

10.0 (SPSS Inc, Chicago, IL, USA) using chi-square for our qualitative variables, and $p < 0.05$ was regarded as significant.

## RESULTS

72% of cases were females and the mean age was 27 years. The mean age of males was 36 years. 51% (179 women) of all specimens belonged to premenopausal women and 21% (74 women) to postmenopausal ones. ER reactivity was seen in 79 cases (22.5%) of all our 351 cases, which comprised 56 (16%) females and 23 (6.5%) males. Positive reactivity was seen in 24% (31/130) of goitres and 21% of neoplastic lesions. In the latter group, we observed ER reactivity in 31% (37/119) of papillary (Fig. 1), 11% (2/18) of follicular, 0% (0/35) of medullary (Fig. 2) and 0% (0/12) of undifferentiated carcinomas, and 22% (8/37) of follicular adenomas.

ER reactivity was seen in 38 (21.2%) cases of premenopausal and 16 cases (21.6%) of postmenopausal women. Three premenopausal women were pregnant, two of them had papillary carcinomas confined to the thyroid and one with goitre, none of their lesions showed ER reactivity. The number of pregnant women in the sample was too few to be of statistical significance. 1.1% (5/42) of normal thyroid tissues showed positive reactivity for ER, including two men with goitre, one postmenopausal woman with papillary carcinoma and one premenopausal woman with medullary carcinoma. Statistical significance was not found between ER reactivity and various thyroid lesions (benign and malignant). Statistical significance was not found between normal thyroid and ER reactivity ($p > 0.1$).

The intensity of staining varied somewhat but all tumours considered to be positive demonstrated distinctly brown or brown-black nuclear staining. The reactivity was confined to the nucleus. No significant differences were found in various age groups, or between women and men, and no statistical significance was found between ER positivity in benign versus malignant lesions. However, in the neoplastic group, there was a significant statistical difference in the proportion with ER positivity between papillary carcinoma versus other thyroid neoplasms ($p < 0.0005$). There were no relationships between ER reactivity and vascular, lymph node and capsular, involvements in the neoplastic group.

## DISCUSSION

Some tumours, such as prostate and breast cancers, are influenced by sex hormones.[1,2] Neoplastic cells possessing hormonal receptors are potentially responsive to the ligands for those receptors. It is well known that thyroid nodules not only occur more frequently in women, they also have a better prognosis. The mechanism of this difference is not clearly understood but it could be related to the exposure to sex hormones. Some studies support this hypothesis and have shown the presence of ERs in thyroid tumours.[1] Growth dependence of some malignant thyroid neoplasms on thyroid stimulating hormone (TSH) is important in therapy, since TSH can be suppressed by using thyroid hormones.[4,7] Recent reports show that ERs are present in thyroid tissue, and suggest that oestrogen may play a role in the biology of thyroid neoplasms.[5-11,18-30] Some studies show that 17-B-oestradiol is a potent mitogen that exerts growth promoting effects not only by binding to nuclear ERs, but also by activating the mitogen-activated proteinkinase pathway.[7]

In this study, we tried to determine whether ER could be detected in neoplastic and nonneoplastic thyroid lesions, and evaluated the difference in ER expression between various age groups, both genders, type of thyroid lesions and invasion capacity. We observed ER reactivity in 24% of goitres, 31% of papillary carcinomas, and 22% of follicular adenomas and also observed strong statistical significance between ER reactivity and papillary carcinomas, compared with other neoplasms ($p < 0.0005$). The absence of ER reactivity in undifferentiated and medullary carcinomas and the relative low proportion in follicular carcinomas, in comparison with goitres, papillary carcinomas and follicular adenomas, suggest that ER reactivity tends to increase with better differentiation of thyroid tumours. Therefore, our results are compatible with those from other studies.[16-18]

Since the incidence of ER reactivity does not significantly differ between females and males, the observed sex differences in thyroid tumour incidence may reflect the higher oestrogen levels in sera in females.[28] However, like other studies done, we found no relation between ER reactivity and age, gender, pregnancy, presence of capsular and vascular invasions, and lymph node status in any tumour.[12] The role of oestrogen activity in thyroid lesions, especially in well-differentiated tumours, such as papillary carcinomas, follicular adenomas and in goitres, merits further investigation.

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