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Original article

# Serum levels of sex hormones and expression of their receptors in thyroid tissue in female patients with various types of thyroid neoplasms

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ABSTRACT

Previous studies have demonstrated the expression of estrogen receptor (ER) and progesterone receptor (PR) in thyroid cancer; however, little is known regarding the levels of estrogen, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in serum and the expression of ER, PR, FSH receptor (FSHR), and LH receptor (LHR) in thyroid tissues of patients with different types of thyroid neoplasms. Serum levels of estrogen, progesterone, FSH, and LH were measured by chemiluminescence, and expression of ER, PR, FSHR, and LHR in thyroid tissue was detected by immunohistochemistry in female patients with thyroid adenoma ($n = 70$), nodular goiter ($n = 73$), thyroid papillary cancer ($n = 149$), poorly differentiated thyroid carcinoma ($n = 12$), or undifferentiated thyroid carcinoma ($n = 8$) and in normal controls ($n = 60$). The positive rates of serum estrogen level and ERα expression were significantly greater in patients with various types of thyroid neoplasms than in normal controls. The positive rates of ERβ expression were significantly less in various types of thyroid neoplasms than in normal thyroid tissues, especially in poorly differentiated carcinoma and undifferentiated carcinoma. The negative rates of serum progesterone level and positive rates of PR expression in thyroid tissue were significantly greater in patients with thyroid adenoma, nodular goiter, or thyroid papillary cancer than in normal controls. The positive rates of serum FSH and LH levels and FSHR and LHR expression were significantly greater in the thyroid adenoma group than in other groups. Our findings suggest that thyroid neoplasms might be sex hormone-dependent. The positive expression of ERα and PR often indicates thyroid papillary carcinoma, and the ERβ expression status is important for the diagnosis of poorly differentiated carcinoma and undifferentiated carcinoma. In addition, thyroid adenoma is often accompanied by an increase in serum FSH and LH levels, as well as FSHR and LHR expression. Thus, the combined detection of serum levels of sex hormones and expression of their receptors allows for a differential diagnosis and evaluation of the degree of differentiation among various types of thyroid neoplasms.



## Introduction

The incidence of thyroid diseases has increased significantly during recent years [1,2]. Over 200 million people worldwide have been affected by various types of thyroid diseases. Thyroid tumors occur more frequently in women than in men, and thyroid cancer frequently shows good differentiation and is associated with a good prognosis in young women [3,4]. Observations that the development of thyroid diseases is related to gender and age have led to the hypothesis that sex hormones may be involved in the development and progression of thyroid diseases [5–7]. It is traditionally believed that estrogen and progesterone function mainly in the breast, uterus, and other so-called "target organs", and that estrogen receptors (ERs) and progesterone receptor (PR) are expressed in the cells of these "target organs"; however, expression of ERs and PR in the liver, thyroid, and other nontraditional "target organs" has been reported in recent years, and numerous studies have shown that ERs and PR have important roles in the pathogenesis of thyroid cancer [8,9].

Despite potentially important roles of estrogen and progesterone and their receptors in thyroid neoplasms, there has been no systematic investigation of serum levels of estrogen and

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progesterone and expression of their receptors in thyroid lesions in patients with various types of thyroid neoplasms. Studies on immunohistochemical localization of ERs and PR in human thyroid tissue have yielded inconsistent results [10–12]. Some studies suggest that ERs and PR exist in both normal and diseased thyroid tissues with different positive expression rates, while others have demonstrated the presence of positive staining for ERs and PR in differentiated thyroid cancer tissue but not in normal or tumor-adjacent tissues [13,14].

In addition to estrogen and progesterone, sex hormones also include follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Currently, it is largely unknown whether serum levels of FSH and LH and expression of FSH receptor (FSHR) and LH receptor (LHR) are significantly altered in various thyroid neoplasms.

The aim of the present study was to investigate the expression of sex hormones and their receptors in various types of thyroid neoplasms. We determined serum levels of estrogen, progesterone, FSH, and LH in female patients with papillary thyroid cancer, poorly differentiated thyroid carcinoma, undifferentiated thyroid carcinoma, thyroid adenoma, or nodular goiter. Thyroid neoplasms can be a benign tumor, such as thyroid adenoma, or a malignant neoplasm, such as papillary thyroid cancer. Currently, there is still controversy regarding whether nodular goiter is neoplastic or hyperplastic. However, given that thyroid neoplasms frequently occur in patients with nodular goiter [15], nodular goiter patients were also included in this study. In addition, we detected the expression of ER, PR, FSHR, and LHR in thyroid lesions from these patients. Investigating the relationship between different sex hormones and their receptors and thyroid neoplasms may provide a new avenue for the diagnosis and treatment of thyroid neoplasms.

## Materials and methods

### *Patients and samples*

Three hundred and twelve female patients surgically treated for thyroid neoplasms at the First Hospital of Jilin University (Changchun, China) from September 2009 to September 2012 were included in this study. Their average age was $37.66 \pm 3.72$ years. Of these patients, 70 had thyroid adenoma, 73 had nodular goiter, 149 had thyroid papillary cancer, 12 had poorly differentiated thyroid carcinoma, and 8 had undifferentiated thyroid carcinoma. No patients received any preoperative radiotherapy or chemotherapy. Serum and thyroid samples were collected from these patients. Sixty samples of normal thyroid tissue adjacent to thyroid adenoma and 60 serum samples from healthy volunteers (age and sex-matched) were also included as normal tissue and serum controls, respectively. All patients provided written informed consent. The study protocol was approved by the Ethics Committee of the First Hospital of Jilin University.

### *Measurements and definitions*

Serum levels of estrogen, progesterone, FSH, and LH were determined by chemiluminescence using commercial kits (Bayer Corporation, Pittsburgh, PA, USA) according to the manufacturer's instructions. Serum levels of estrogen, FSH, and LH greater than normal levels (http://www.bayerus.com/) were defined as positive. A preliminary experiment demonstrated that serum levels of progesterone were not elevated in any of the patient groups, but they were less in many patients, compared to normal levels. Thus, serum levels of progesterone less than the normal range were defined as negative. The normal range of various sex hormone levels in serum of females is listed in Table 1. It is worth noting that in a few non-positive cases (Estrogen: 2/312; Progeterone: 1/312; FSH: 8/312; LH: 4/312), serum hormone levels were not in the normal range. Since this study did not focus on them, they were not described further.

**Table 1**
The normal range of various sex hormone levels in serum of females.

| Hormone | Menopause | Follicular phase | Luteal phase |
|---|---|---|---|
| Estrogen (pmol/L) | 73.4–146.8 | 99.09–447.7 | 178.83–1068 |
| Progesterone (nmol/L) | 0.25–2.48 | 0.99–4.83 | 16.41–59.02 |
| FSH (mIU/mL) | 16.74–113.6 | 3.85–8.78 | 1.79–5.12 |
| LH (mIU/mL) | 10.87–58.64 | 2.12–10.89 | 1.20–12.86 |

FSH, follicle-stimulating hormone; LH, luteinizing hormone.

### *Immunohistochemistry*

Tissue specimens were fixed in 10% formaldehyde, paraffin-embedded, sectioned into 3-μm thick serial sections, and subjected to immunohistochemical staining using commercial kits according to the manufacturer's instructions. ER and PR kits were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA), while FSHR and LHR kits were provided by Boster Biological Technology (Wuhan, China). Cells positive for ER, PR, FSHR, or LHR were those showing brown staining in the cytoplasm or nucleus that was significantly greater than background levels. Five high-magnification (400×) fields were selected randomly to count the number of positive cells in various types of tissues. Tissues in which the percentage of positive cells was greater than 25% were defined as positive (+).

### *Statistical analysis*

Statistical analyses were performed using SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). Categorical data were expressed as percentages and compared using the chi-squared test. *P*-values <0.05 were considered statistically significant.

## Results

### *Levels of estrogen in serum and expression of ER in thyroid tissue*

As shown in Table 2, the positive rates of serum estrogen level were significantly greater in patients with various types of thyroid neoplasms (except undifferentiated carcinoma) than in normal controls ($P < 0.05$). However, there were no significant differences in the positive rates of serum estrogen level among patients with various types of thyroid neoplasms ($P > 0.05$).

ERs were distributed in both the cytoplasm and nuclei of thyroid epithelial cells. ERα expression was detected in three types of thyroid neoplasms and normal thyroid tissue. The positive rates of ERα expression were significantly greater in papillary carcinoma than in thyroid adenoma, normal thyroid and thyroid nodular goiter ($P < 0.01$) (Table 3, Fig. 1). ERβ expression was detected in all types of thyroid neoplasms and normal thyroid tissue. The positive rates of ERβ expression were significantly less in various types of thyroid neoplasms than in normal thyroid tissue, especially in poorly differentiated carcinoma and undifferentiated carcinoma ($P < 0.01$) (Table 3, Fig. 2).

### *Levels of progesterone in serum and expression of PR in thyroid tissue*

The negative rates of serum progesterone level were significantly greater in patients with thyroid adenoma, nodular goiter, low differentiated carcinoma, or thyroid papillary cancer than in normal controls ($P < 0.05$) (Table 2). There were no significant