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Relationship of thyroid ultrasound elasticity contrast index with serum autoantibody and Th1/Th2 cytokine levels in patients with Hashimoto´s thyroiditis

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ABSTRACT

Objective: To study the relationship of thyroid ultrasound elasticity contrast index (ECI) with serum autoantibody and Th1/Th2 cytokine levels in patients with Hashimoto's thyroiditis. **Methods:** A total of 68 patients diagnosed with Hashimoto's thyroiditis (HT) in our hospitalas were selected as HT group, 60 healthy volunteers were selected as control group, ultrasound examination was performed to determine ECL, serum was collected to determine TPO-Ab, TG-Ab, IFN- γ , TNF- α , IL-2, IL-4 and IL-10 levels, and peripheral blood was collected to determine the positive expression rate of CD30 and CD195. **Results:** ECI of HT group was significantly higher than that of control group and the ECI of patients with small nodule type HT was significantly lower than that of patients with grid type HT; TPO-Ab, TG-Ab, IFN- γ , TNF- α and IL-2 levels in serum and positive expression rate of CD195 in peripheral blood of HT group were significantly higher than those of control group while IL-4 and IL-10 levels and positive expression rate of CD30 in peripheral blood were significantly lower than those of control group; TPO-Ab, TG-Ab, IFN- γ , TNF- α and IL-2 levels in serum and positive expression rate of CD195 in peripheral blood of patients with small nodule type HT were significantly lower than those of patients with grid type HT while IL-4 and IL-10 levels and positive expression rate of CD30 in peripheral blood were significantly higher than those of patients with grid type HT; ECI was positively correlated with TPO-Ab, TG-Ab, IFN- γ , TNF- α , IL-2b and CD195, and negatively correlated with IL-4, IL-10 and CD30. **Conclusions:** ECL significantly increases in patients with Hashimoto's thyroiditis and it can be used to evaluate the degree of immune dysfunction.

1. Introduction

Hashimoto's Thyroiditis (HT), also called chronic lymphocytic thyroiditis, belongs to the category of autoimmune thyroid disease (AITD). Massive lymphocyte infiltration and the generation of autoantibody such as anti-thyroid peroxidase antibody (TPO-Ab) antibodies and anti-thyroglobulin antibody (TG-Ab) within thyroid tissue are the important pathological features of the disease, and can cause thyroid tissue structure damage and thyroid hypofunction. HT is with hidden onset and slow illness development, only shows goiter in early stage, lacks other clinical manifestations, and is not conducive to early diagnosis. The anatomical position of thyroid is superficial, color Doppler ultrasound is the preferred auxiliary examination method for diagnosis of thyroid diseases[1], and ultrasound elasticity contrast index (ECI) is the difference of tissue strain determined by elasticity imaging, and can reflect the uniformity of strain in tissue[2,3]. In the following study, the relationship of thyroid ultrasound elasticity contrast index with serum autoantibody and Th1/Th2 cytokine levels in patients with Hashimoto's thyroiditis was analyzed.

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2. Materials and methods

2.1. Research subjects

A total of 68 patients diagnosed with HT in our hospital from May 2014 to December 2015 were included in Hashimoto's thyroiditis group (HT group) of the study, and all patients were with the clinical symptoms of diffuse goiter and the elevated TPO-Ab and/or TG-Ab confirmed through laboratory examination, conformed to the diagnosis of Hashimoto's thyroiditis, and never received antithyroid therapy or hormone replacement therapy. 60 cases of healthy volunteers who received physical examination with in our hospital during the same period were selected as the control group of the study. HT group included 24 male cases and 44 female cases who were (46.2±6.4) years old; control group included 25 male cases and 35 female cases who were (44.3±5.9) years old. Comparison of general data between two groups showed no significant difference.

2.2. Research methods

2.2.1. Thyroid ultrasound elasticity contrast index detection methods

Color Doppler ultrasonic diagnostic instrument was used for testing, the probe was with the frequency of 3-12 MHz and elasticity imaging function, and the test process was sketched as follows: patients took supine position and exposed anterior cervical region, the head was slightly backward to make the neck at full stretch, the ultrasonic probe was used to routinely scan bilateral lobe and isthmus of the thyroid and judge the type of the thyroid gland, including diffuse small nodule type and diffuse grid type, and the thyroid volume, internal echo and blood flow signal were recorded; long axis of thyroid was selected, patients were told to hold breath for 3-5 s and not to swallow, it was saved after all the color bar showed stable green images, and the ultrasound ECI was determined in region of interest (ROI).

2.2.2. Serum sample collection and detection methods

A total of 5 mL of fasting peripheral venous blood was collected from HT group and healthy controls before ultrasound examination, let stand for 20 min at room temperature and then immediately centrifuged in 4 ℃ centrifugal machine for 15 min at the speed of 3 000 r/min, serum was separated and enzyme-linked immunosorbent assay kit was used to detect TPO-Ab, TG-Ab, IFN-γ, TNF-α, IL-2, IL-4 and IL-10 levels.

2.2.3. Peripheral blood sample collection and detection methods

Peripheral venous blood samples collected before ultrasound examination were taken, diluted with same volume of PBS solution, then added to lymphocyte separation medium and centrifuged, lymphocytes in the middle layer were extracted, FITC-labeled CD30 antibodies and PE-labeled CD195 were incubated respectively, and 2 hours later, positive expression rate of CD30 and CD195 was determined in flow cytometer.

2.3. Statistical methods

SPSS20.0 software was used to input and analyze data, measurement data analysis between two groups was analyzed by *t* test, correlation analysis between two data was by Pearson test and $P<0.05$ indicated statistical significant differences.

3. Results

3.1. Thyroid elasticity imaging and ECI value

ECI of HT group measured by thyroid elasticity imaging was 2.94±0.51; ECI of control group measured by thyroid elasticity imaging was 1.12±0.25. After statistical analysis, ECI of HT group was significantly higher than that of control group. ECI of patients with small nodule type HT was 2.28±0.35, ECI of patients with grid type HT was 3.52±0.61, and after statistical analysis, the ECI of patients with small nodule type HT was significantly lower than that of patients with grid type HT.

3.2. Serum autoantibody TPO-Ab and TG-Ab levels

Serum autoantibody TPO-Ab and TG-Ab levels of HT group were significantly higher than those of control group; serum autoantibody TPO-Ab and TG-Ab levels of patients with small nodule type HT and grid type HT were significantly higher than those of control group, serum autoantibody TPO-Ab and TG-Ab levels of patients with small nodule type HT were significantly lower than those of patients with grid type HT (Table 1).

Table 1
Serum TPO-Ab and TG-Ab levels (IU-mL).

Groups	Case No.	TPO-Ab	TG-Ab
HT group	68	295.51±38.74*	214.59±36.58*
Small nodule type	32	221.37±32.58*	142.21±20.38*
Grid type	36	378.14±55.26*a	291.35±48.43*a
Control group	60	20.38±4.28	14.27±2.92

*: compared with control group, $P<0.05$; a: compared between small nodule type and grid type, $P<0.05$.

3.3. Serum Th1/Th2 cytokine levels

Serum IFN-γ, TNF-α and IL-2 levels of HT group were higher than those of control group while IL-4 and IL-10 levels were lower than those of control group; serum IFN-γ, TNF-α and IL-2 levels of patients with small nodule type HT and grid type HT were higher than those of control group while IL-4 and IL-10 levels were lower

Table 2
Serum Th1/Th2 cytokine levels.

Groups	Case No.	Th1 cytokines			Th2 cytokines	
		IFN- γ (ng/L)	TNF- α (ng/L)	IL-2 (μg/L)	IL-4 (ng/L)	IL-10 (ng/L)
HT group	68	214.4±33.5*	132.8±16.8*	14.91±2.17*	104.5±16.8*	58.7±7.9*
Small nodule type	32	152.2±19.8*	89.5±10.4*	11.38±1.48*	145.5±17.9*	74.2±9.3*
Grid type	36	289.7±40.7*a	179.4±25.2*a	19.04±2.75*a	70.3±9.3*a	35.5±4.2*a
Control group	60	89.3±11.4	55.3±7.8	6.27±0.81	240.2±35.8	118.4±17.9

*: compared with control group, $P<0.05$; a: compared between small nodule type and grid type, $P<0.05$.

than those of control group; serum IFN- γ , TNF- α and IL-2 levels of patients with small nodule type HT were significantly lower than those of patients with grid type HT while IL-4 and IL-10 levels were significantly higher than those of patients with grid type HT (Table 2).

3.4. CD30 and CD195 expression levels in peripheral blood

Positive expression rate of CD195 in peripheral blood of HT group was significantly higher than that of control group while positive expression rate of CD30 was significantly lower than that of control group; positive expression rate of CD195 in peripheral blood of patients with small nodule type HT and grid type HT were significantly higher than those of control group while positive expression rate of CD30 were significantly lower than those of control group; positive expression rate of CD195 in peripheral blood of patients with small nodule type HT was significantly lower than that of patients with grid type HT while positive expression rate of CD30 was significantly higher than that of patients with grid type HT (Table 3).

Table 3
CD30 and CD195 expression levels in peripheral blood.

Groups	Case No.	CD195	CD30
HT group	68	38.69±5.12*	9.14±1.16*
Small nodule type	32	34.22±4.48*a	11.35±1.35*a
Grid type	36	43.08±6.28*	7.68±0.93*
Control group	60	28.39±3.49	15.29±2.03

*: compared with control group, $P<0.05$; a: compared between small nodule type and grid type, $P<0.05$.

3.5. Correlation of ECI with serum indexes and peripheral blood indexes

Pearson correlation analysis showed that ECI was positively correlated with TPO-Ab, TG-Ab, IFN- γ , TNF- α and IL-2 levels in serum and positive expression rate of CD195 in peripheral blood, and correlation coefficient r were 0.714, 0.663, 0.732, 0.626, 0.648 and 0.702 respectively; ECI was negatively correlated with IL-4 and IL-10 levels in serum and positive expression rate of CD30 in peripheral blood, and correlation coefficient r were -0.596, -0.751 and -0.628 respectively.

4. Discussion

HT is with relatively hidden condition and only manifested as elevated autoantibody TPO-Ab and TG-Ab levels, thyroid function is not yet obviously damaged and is within the normal range, and then the damage of the thyroid follicular cells will cause thyroid hyperfunction, eventually developing into hypothyroidism. The anatomical position of thyroid is superficial, and color Doppler ultrasound can obtain clear tissue images and identify different diseases[4,5]. Diffuse small nodule type HT and diffuse grid type HT are two most common ultrasonic findings of HT; the essence of the former still belongs to pseudotubercle, it is the change caused by focal or scattered infiltration of lymphocytes in thyroid parenchyma, and the pathological changes of thyroid tissue at this time are mostly lighter; the latter is that there are different degree of fibrous tissue proliferation and fibrous septum in thyroid tissues, and the pathological changes of thyroid tissue at this time are mostly more severe[6,7].

The judgment of diffuse small nodule type and diffuse grid type HT is greatly affected by the subjective judgment of the operators, and the clinical ultrasonic quantitative indexes that can accurately judge the severity of HT are still short at present. ECI is an ultrasonic quantitative index developed on the basis of ultrasonic elastography[8], avoids the limitation of the traditional triggered elastography, and can determine the tissue hardness as well as the difference and homogeneity of all pixel strain in the ROI at the same time[9,10]. In the study, comparison of thyroid ECI between HT patients and healthy subjects confirmed that ECI of HT group was significantly higher than that of control group and the ECI of patients with small nodule type HT was significantly lower than that of patients with grid type HT. It means that ECI can be used in identification of HT patients and healthy subjects, and meanwhile, it can also be used as the quantitative indicator to assess thyroid elasticity of HT patients.

HT belongs to the category of autoimmune disease, and the body's immune dysfunction and immune tolerance damage are the key link causing the occurrence of disease[11]. Autoantibody TPO-Ab and TG-Ab are the main elements causing the thyroid function damage, and serum TPO-Ab and TG-Ab levels are also the main indexes to diagnose diseases and assess their conditions. TG is the iodide diabetes synthesized and secreted by thyroid epithelial cells, is the precursor synthesized by T3 and T4, and is stored in the thyroid follicle; TG-Ab can be combined with TG and activate NK cells, causing the thyroid follicular cell damage[12]. TPO is an enzyme that

is synthesized by thyroid follicular cells and can catalyze the thyroid hormone synthesis, and TPO-Ab will influence the activity of TPO and lead to deficiency of thyroid hormone synthesis and thyroid follicular cell damage[13]. In the study, analysis of serum TPO-Ab and TG-Ab levels in patients with HT showed that serum TPO-Ab and TG-Ab levels of HT group were higher than those of control group, serum TPO-Ab and TG-Ab levels of patients with small nodule type HT were lower than those of patients with grid type HT and serum TPO-Ab and TG-Ab levels were positively correlated with ECI. This means that the ECI can evaluate the immune dysfunction extent and disease severity in patients with HT.

$CD4^+$T lymphocytes are the important helper cells in the body's immune system and are mainly involved in the autoimmune response mediated by cellular immune response, and abnormality of $CD4^+$T cell levels can cause the disorder of immune function and the destruction of immune tolerance[14,15]. Both Th1 and Th2 in helper T cells are the important subsets of $CD4^+$T cells, the former main secretes IFN-γ, TNF-α and IL-2 and can promote further proliferation of Th1, and the latter mainly secretes IL-4 and IL-10 and can inhibit Th1 activation and proliferation[16,17]. Th1/Th2 balance shifting to Th1 is an important part of the HT attack[18], the determination of serum levels of Th1 and Th2 cytokines in the study showed that serum IFN-γ, TNF-α and IL-2 levels of HT group were higher than those of control group and positively correlated with ECI while IL-4 and IL-10 levels were lower than those of control group and negatively correlated with ECI. CD195 and CD30 are the specific factors on $CD4^+$T cell surface and are also the dominant molecules on Th1 and Th2 surface respectively, analysis of the positive expression rate of CD195 and CD30 in peripheral blood lymphocytes in the study proved that the CD195 expression level in peripheral blood of HT group was higher than that of control group and positively correlated with ECI while CD30 expression rate was lower than that of control group and negatively correlated with ECI. It means that ECI has evaluation value for the degree of Th1/Th2 immune balance disorder in patients with HT.

To sum up, ECL significantly increases in patients with Hashimoto's thyroiditis and it can be used to evaluate the degree of immune dysfunction.

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