ORIGINAL ARTICLE

Anti-livin antibodies in Hashimoto thyroiditis

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KEY WORDS

anti-livin antibodies, Hashimoto thyroiditis

ABSTRACT

INTRODUCTION Livin belongs to the family of apoptosis inhibitors. High livin expression is observed in malignancies of the gastrointestinal tract, lungs, breast, and kidneys, but it is not present in differentiated adult tissues. In some malignant processes, anti-livin antibodies are present.

OBJECTIVES The aim of the study was to evaluate the prevalence of anti-livin antibodies in Hashimoto thyroiditis, a disease characterized by rapid and widespread thyrocyte apoptosis.

PATIENTS AND METHODS The study comprised 65 women with Hashimoto thyroiditis and the control group of 40 healthy women. In the majority of the patients, clinical manifestations of hypothyroidism were observed; all patients had high levels of serum antithyroid peroxidase antibodies. A solid-phase radioimmunoassay in livin-coated polyethylene tubes using 125I-labeled protein A was used to determine anti-livin antibodies.

RESULTS Significant amounts of anti-livin antibodies were reported in 18 patients (26.8%); 3 patients (4.6%) had borderline antibody levels; while in controls only 1 patient was positive (2.5%, $P < 0.0001$).

CONCLUSIONS In Hashimoto thyroiditis, an autoimmune process is more general and involves numerous autoantibodies including an antibody against apoptosis inhibitor – livin. Anti-livin antibodies cannot serve only as a marker of malignancy because they are also present in autoimmune processes.

INTRODUCTION Livin belongs to the family of inhibitors of apoptosis (IAP). So far, 8 apoptosis inhibitors have been described in humans: baculoviral IAP repeat-containing proteins 2 and 3, neuronal apoptosis inhibitory protein, survivin, X-linked inhibitor of apoptosis protein, Bruce, ILP-2, and livin.[1-3] Proteins of this family occur in all mature cells of the human body. Exceptions are livin and survivin, which are found only in fetal tissues. High expression of livin and survivin is observed in intestinal cancer, breast cancer, and other malignancies.

Livin was first discovered and described by Vucic et al.[4] in malignant melanoma. Further detailed studies on livin were conducted by Kasof and Gomez[5], who named this inhibitor livin.[5] They studied the amino acid sequence of livin and concluded that it consisted of 70 amino acids homologically resembling the sequence of baculoviruses and other apoptosis inhibitors. Livin affects apoptosis, inhibits cell cycles, affects proliferation, persistence and progression of cancer cells.[6] Destruction and apoptosis of thyroid cells occur also in Hashimoto disease, which is characterized by excessive inflammatory and immune reactions.

The aim of our study was to evaluate whether inflammation of thyroid tissue and intensive thyrocyte apoptosis in Hashimoto thyroiditis are associated with the presence of anti-livin antibodies.

PATIENTS AND METHODS The study included 65 women with Hashimoto thyroiditis, aged from 21 to 63 years. Diagnosis of Hashimoto thyroiditis was based on 3 main findings: the presence of goiter, typical ultrasonography, and elevated anti-thyroid peroxidase antibodies (anti-TPO). Twenty-seven patients were diagnosed during hospitalization at the Department of Endocrinology, Metabolism and Internal Diseases, Poznan University of Medical Sciences, Poznań, Poland, while the remaining patients were observed and treated at an outpatient clinic. Thirty-eight patients, including those on substitution therapy, had symptoms of hypothyroidism. Three patients had thyrotoxicosis (Hashitoxicosis). Some patients had enlarged, tender thyroid while others had nodular goiters. Clinical manifestation of hypothyroidism varied between patients. The majority of patients had already been on substitution

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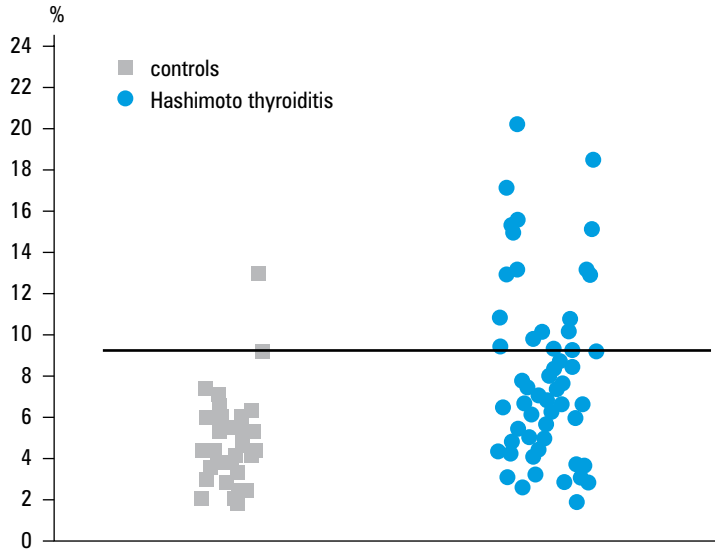

FIGURE Anti-livin antibody levels in patients with Hashimoto thyroiditis and in control subjects

therapy with L-thyroxine prescribed by a family doctor; some patients were evaluated at the early stage of the disease and had only mild symptoms. Typical clinical manifestations included easy fatigue, sleep disorders, weight gain, deficient memory, and menstrual irregularities (in reproductive women). Physical examination revealed mainly dry skin, puffy face and hands, dry and matt hair, bradycardia, and hypertension. Neck ultrasonography showed hypoechogenic thyroid with some longitudinal hyperechogenic streaks or thyroid with irregular hypoechogenic patches. Four patients with hypothyroidism after radioiodine treatment due to Grave's disease had ophthalmopathy.

In all patients, thyroid-stimulating hormone (TSH), free triiodothyronine (FT_3), free thyroxine (FT_4), anti-TPO, and antithyroglobulin antibodies (ATA) were determined. Patients who had not been treated previously and had clinical manifestations of hypothyroidism had higher TSH (21–83 mU/l; normal range, 0.27–4.2 mU/l), lower FT_4 (2.2–6.4 pml/l; normal range, 11.5–21 pmol/l), and lower FT_3 (0.9–3.5 pmol/l; normal range, 3.93–7.7 pmol/l). In patients with Hashitoxicosis, TSH could not be detected, while FT_4 and FT_3 were increased. Normal TSH, FT_4, and FT_3 were observed in patients treated with L-thyroxine before the examination. In patients with Grave's ophthalmopathy, TSH receptor antibodies were elevated. In all patients, anti-TPO were significantly increased and ranged between 157 and 3000 IU/ml. In 7 patients, Hashimoto thyroiditis was associated with other endocrinopathy (Addison's disease or diabetes).

The control group comprised women matched for age without past or present thyroid diseases.

Materials Livin was purchased from R&D Systems; protein A and other chemicals from SIGMA and GE Healthcare; iodine-125 (^{125}I) isotope and polyethylene tubes from OBRI Świerk (Poland).

Blood samples were taken from the antecubital veins into polyethylene tubes. We used a solid-phase radioimmunoassay to determine anti-livin antibodies and previous adsorption studies to test antibody specificity. A radioimmunoassay for anti-livin antibodies was described in detail in our previous paper.[11] Briefly, polypropylen tubes coated with livin (1 ug/tube) were incubated with diluted patient's sera. Next day, the tubes were decanted, washed, and ^{125}I-labeled protein A was added and incubated overnight. Afterwards, the tubes were decanted, washed, and their activity measured in γ-counter (LKB Wallac, Finland).

Statistical analysis The results of the radioimmunoassay were compared with the results of healthy controls. Because of nonparametric data distribution, we used the nonparametric Mann-Whitney *U* test. Statistical significance was set at a *P* level of less than 0.05.

RESULTS The results exceeding 2 standard deviations of the reference value calculated in the group of control subjects were considered positive. Anti-livin antibodies were detected in 18 patients (26.8%), of whom 3 patients had borderline antibody levels. Only 1 subject was positive for anti-livin antibodies in the control group. The difference between the study and the control group was statistically significant ($P < 0.01$) (FIGURE).

DISCUSSION Currently, there are no publications on anti-livin antibodies in autoimmune diseases available in the literature. There have only been papers on the excessive expression of livin and survivin in various types of cancers. The presence of anti-livin antibodies was for the first time detected by Yagihashi et al.[7] in patients with malignancies of the intestinal tract and later in those with breast and lung cancer.[8-10] The authors used an enzyme immunoassay to detect antibodies, a similar method to our radioimmunoassay described previously.[11] The specificity of our radioimmunoassay was evaluated by SDS PAGE and Western blot and was found to be satisfactory.[11]

Botazzo et al.[12] used immunofluorescence and flow cytometry to analyze the presence and expression of Bcl02 and Apo-I/fas in thyrocytes of patients with Hashimoto thyroiditis. The authors observed increased apoptosis in thyrocytes.[12] The expression of Fas was also evaluated in Hashimoto thyroiditis by Bossowski et al.[13] The authors concluded that alteration in the expression of proapoptotic proteins in thyroid follicular cells may play a role in the pathogenesis of thyroid autoimmune disorders. A defect in CD4(+) and CD25(+) T regulatory cells breaks immune tolerance in Hashimoto thyroiditis. T-helper cells type 1 stimulate macrophages and cytotoxic lymphocytes and secrete interleukin (IL) 2, tumor necrosis factor-β, interferon γ, and several cytokines.[4-6,10,13] These proteins increase the production of IL-12. T-helper

cells type 2 activate and direct B cells to produce anti-TPO and, in some cases, ATA.[14,15]

We have been studying autoimmune processes in patients with hypothyroidism at our department for many years. Initially, we isolated thyroglobuline in microsomal fraction from human thyroid glands and introduced a radioimmunoassay for these protein. This allowed us to detect ATA in Hashimoto disease[16] and later antimicrosomal antibodies.[17] Subsequent studies involved isolation of tubulin, a cytoskeletal protein from human brains, and its use in a solid-phase radioimmunoassay.[18] In Hashimoto disease, high prevalence of antitubulin antibodies was observed.[18] Next, we observed the presence of antibodies to several muscle proteins (actin, myosin, myoglobin, troponin, and tropomyosin).[19]

A large group of patients with circulating antitriiodotyronine and antithyroxine autoantibodies have been studied by Ruchała et al.[20] In these cases, we encountered severe diagnostic difficulties because these antibodies disturb thyroid hormone assays. Most patients described by Ruchała et al.[20] had Hashimoto thyroiditis. Abaci et al.[21] reported increased prevalence of anticardiolipin antibodies in patients with autoimmune thyroiditis.

The above studies suggest that autoimmune process in Hashimoto thyroiditis is systemic and involves several antigens, including livin, the apoptosis inhibitor. Our studies indicate that anti-livin antibodies cannot serve only as a marker of malignancies because they also occur in autoimmune processes.

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ARTYKUŁ ORYGINALNY

Przeciwciała antyliwinowe w chorobie Hashimoto

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SŁOWA KLUCZOWE

choroba Hashimoto, przeciwciała antyliwinowe

STRESZCZENIE

WPROWADZENIE Liwina należy do inhibitorów apoptozy komórek. Jej wysoką ekspresję stwierdza się w komórkach nowotworów przewodu pokarmowego, płuc, piersi i nerek, natomiast nie występuje w zróżnicowanych tkankach osób dorosłych. W niektórych procesach złośliwych pojawiają się przeciwciała antyliwinowe.

CELE Celem pracy było zbadanie obecności przeciwciał antyliwinowych w zapaleniu tarczycy typu Hashimoto, ponieważ w chorobie tej dochodzi do gwałtownej i masowej apoptozy tyreocytów.

PACJENCI I METODY Badania objęły 65 kobiet z chorobą Hashimoto oraz grupę kontrolną 40 zdrowych kobiet. U większości chorych występowały kliniczne objawy niedoczynności tarczycy; u wszystkich chorych stwierdzono wysoki poziom przeciwciał przeciwko peroksydazie tarczycowej. Przeciwciała antyliwinowe oznaczano metodą radioimmunologiczną fazy stałej w probówkach opłaszczonych liwiną z zastosowaniem białka A znakowanego ^{125}I.

WYNIKI Przeciwciała antyliwinowe w istotnych ilościach stwierdzono u 18 chorych (26,8%); u 3 chorych (4,6%) ich poziomy znajdowały się na pograniczu normy, natomiast w grupie kontrolnej wystąpiły tylko w 1 przypadku (2,5%, $p < 0,0001$).

WNIOSKI W chorobie Hashimoto proces autoimmunizacyjny jest bardziej uogólniony i występuje w nim wiele autoprzeciwciał, w tym przeciwko inhibitorowi apoptozy – liwinie. Przeciwciała antyliwinowe nie mogą być postrzegane tylko jako marker procesów nowotworowych, ponieważ są obecne również w procesach autoimmunologicznych.

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