ABSTRACT

Introduction

Autoimmune diseases are a common and significant health problem, which incidence in population increase. They affect 3- 5% of the general population, mainly females. Endocrine disorders comprise approximately 30% of all autoimmune disorders. Among paediatric patients are the most common: autoimmune thyroid disease (AITD) and type 1 diabetes mellitus (T1DM). AITD and T1DM are known from their prevalence and possibility of co-occurrence with each other and other autoimmune diseases.

The pathogenesis of AITD involves a loss of immune tolerance to own antigens, manifested by lymphocytic infiltration, damage to the thyroid gland and the production of specific autoantibodies. There are two major forms of AITD: Hashimoto's thyroiditis (HT) and Graves' disease. Well-known markers of thyroid autoimmunity are antibodies against thyroid peroxidase (TPOAb), thyroglobulin (TGAb) and the receptor for TSH (TRAb). They are important diagnostic and prognostic tool in medical practice.

A potential antigen in thyroid tissues is the zinc transporter 8 ZnT8, a member of the SIC30A8 family. Zinc is an important cellular component, with a role in gene expression, cellular metabolism and the catalysis and regulation of biological processes. Cytoplasmic homeostasis of zinc is regulated by zinc transporter proteins, which expression has been detected in endocrine cells, including the thyroid gland. In AITD is observed higher incidence of zinc transporter 8 autoantibodies (ZnT8Ab). The role of ZnT8Ab as a marker of T1DM is well known, as glutamic acid decarboxylase autoantibodies (GADAb), protein tyrosine insulinoma antigen 2 autoantibodies (IA2Ab) and insulin autoantibodies (IAb). All of them may appear prior to the clinical manifestations of T1DM.

The similar aetiology of AITD and T1DM and their relevance in the paediatric population invite to further observations. There are a lot of studies on the presence and role of anti-thyroid antibodies in patients with T1DM. The studies about ZnT8Ab and other islet cells autoantibodies in patients with AITD are rare. Early detection of the initial phase of T1DM is important due to the possibility pf preventing complications, such as ketoacidosis. The results of this study will allow to estimate the risk of T1DM in patients with AITD, improve diagnosis and introduce screening and preventive measures for these patients.

Aim of the study

The main aim of the study was to assess the prevalence of ZnT8Ab in children with AITD. The study was extended to analyse the presence of other islet cell autoantibodies: GADAb, IA2Ab, IAb.

Material and methods:

The study group consisted of patients from the Department of Paediatrics, Endocrinology, Diabetology and Cardiology, Medical University of Bialystok and the Endocrinology Outpatient Clinic of the University Children's Clinical Hospital in Bialystok. A total study consisted of 425 subjects (262 girls, 163 boys, mean age 12.93 ± 3.87 years). The three main groups included: I- 135 children diagnosed with AITD without T1DM, II- 212 children with T1DM with and without AITD, III- control group, 78 children without autoimmune, inflammatory diseases and no autoimmune family history. 63 patients with AITD and T1DM were selected from group II for further analysis. AITD group consisted of 135 children, 72 with HT and 63 with GD, 23 boys and 112 girls, mean age 14.01 ± 3.03 years, mean AITD duration 1.89 ± 2.06 years. AITD onset was found in 33 patients. The AITD and T1DM group consisted of 63 patients, 54 with HT and 9 with GD, 13 boys and 50 girls, mean age 12.93 ± 4.12 years (2-18 years), mean T1DM duration 5.72 ± 4.01 years and AITD duration 2.07 ± 2.84 years. A new diagnosis of AITD was made in 23 of them. The control group consisted of 78 children, 42 boys and 36 girls, mean age 12.67 ± 3.72 years. The study included: anthropometric data, age, gender, duration and form of the disease, coexistence of another autoimmune diseases. Data was obtained by physical examination and from medical records.

In the hospital laboratory serum levels of thyrotropin (TSH), free thyroxine (fT4), free triiodothyronine (fT3), TPOAb, TGAb, TRAb were measured by electrochemiluminescence immunoassay using Cobas e 411 analyser (Roche Diagnostics, Poland). ZnT8Ab, GADAb were determined by enzyme-linked immunosorbent assay (ELISA) using kits from RSR Ltd., (Cardiff, UK). IA2Ab and IAb were measured by immunoprecipitation assay (IPA) using kits from RSR Ltd. (Cardiff, UK). The results were considered positive as suggested in the kit instructions.

Statistical analysis of the determinations made was performed using Statistica 13.0 software (Stat Soft Krakow).

Results

ZnT8Ab were found in 7.41% (n=10) patients with AITD, 7.94% (n=5) with GD and 6.94% (n=5) with HT. In the group of children with AITD and T1DM, they were reported in 61.9% (n=39) subjects, 55.56% (n=5) with GD and 62.96% (n=34) with HT. In the control group they were present in 2.56% (n=2) subjects. Patients with AITD and T1DM were the most positive for ZnT8Ab compared to the other groups. There was a clear trend of a higher prevalence of ZnT8Ab in children with AITD, HT and GD, but not statistically significant to the control group. There were no statistically insignificant differences in the prevalence of ZnT8Ab in children with HT and GD

There was a higher prevalence, although not significantly, of ZnT8Ab in new diagnosed children compared to those previously treated - in AITD: 9.09% (n=3) and 6.86% (n=7), respectively in AITD and T1DM: 66,57% (n=16) and 57,5% (n=23).

None of the study groups showed statistically significant correlations between the presence of ZnT8Ab and age group or gender.

GADAb were found in 5.19% (n=7) children with AITD: 4.17% (n=3) with HT and 6.35% (n=4) with GD; 68.25% (n=43) subjects with AITD and T1DM: 77.78% (n=7) with GD and 66,67% (n=36) with HT and 2.56% (n=2) in the control group. There was a statistically significant highest prevalence of GADAb in children with AITD and T1DM compared to other groups. The differences between GADAb incidence in AITD group and the control group were not significant.

IA2Ab were found in 0.74% (n=1) patients with AITD, 22.22% (n=14) with AITD and T1DM and none in the control group. The prevalence of IA2Ab was statistically significantly highest in children with coexisting T1DM.

IAb were demonstrated in 2.22% (n=3) patients with AITD, 1.59% (n=1) with GD and 2.78% (n=2) with HT. The prevalence of IAb was found to be highest in subjects with AITD and T1DM: 88.89% (n=59), 90.74% (n=49) with HT and 77,78% (n=7) with GD.

There was no significant effect of gender, age group membership, moment of thyroid disease diagnosis on the prevalence of GADAb, IA2Ab, IAb in children with AITD. As well as there were no significant differences in their prevalence in children with HT and GD.

At least one of the tested antibodies was found in 10.37% (n=14) subjects with AITD, 9.72% (n=7) in HT and 11.11% (n=7) in GD. Single antibodies, ZnT8Ab or GADAb, were found in nine patients with AITD. Three patients were positive for two types of antibodies, while two children for three. The most frequently observed was coincidence of ZnT8Ab and GADAb and ZnT8Ab and IAb.

In children with T1DM there were no significant differences in the prevalence of ZnT8Ab, GADAb, IA2Ab according to the co-occurrence of AITD. There was a statistically significant higher prevalence of IAb in patients with AITD in relation to those without AITD. At least one type of antibody was shown in all children with AITD and T1DM and 96.64% in T1DM without AITD. Respectively 82.54% and 83.89% were positive for two, and 47.62% and 49.66% for three and more types. There was no significant disparity between the groups in the co-occurrence of different antibodies, either in number or type.

In children with AITD and T1DM there were no statistically significant differences in the prevalence of ZnT8Ab, GADAb, IA2Ab, IAb according to age group, gender, time of thyroid disease diagnosis, or between subjects with GD and HT.

Children from control group were positive for at least one of the tested antibodies in 5.13% (n=4). The most prevalent were ZnT8Ab and GADAb, each in 2.56% (n=2) subjects. Age and gender were not significant for their prevalence. No IA2Ab and IAb or co-occurrence of different types of antibodies was found.

In the entire study group of children with AITD, the co-occurrence of other autoimmune diseases was observed in 41.42% (n=83) of 198 children, as determined by group selection. All subjects with AITD and T1DM were included. 14.81% (n=20) of patients with AITD without Ct1 had an additional autoimmune disease. T1DM was most frequently noted in 31.82% (n=63), followed by coeliac disease- 10.6% (n=21), vitiligo- 5.56% (n=11), Addison's disease (AD)- 2.02% (n=2). Less frequently observed were: alopecia areata - 1.52% (n=3), juvenile idiopathic arthritis (JIA) - 1.01% (n=2), psoriasis - 1.01% (n=2), essential thrombocytopaenia - 1.01% (n=2), myasthenia gravis (MG) - 0.51% (n=1).

A statistically significantly more frequent coexistence of other autoimmune diseases was observed in the oldest age group (11-18 years) of the examined children with AITD, no such relationship was found with gender. Children with HT were significantly more likely to have other autoimmune disease than with GD, and their presented a higher number of additional autoimmune diseases, with the most frequent T1DM.

In children with AITD with another autoimmune disease there were a statistically significant higher frequency of ZnT8Ab, GADAb, IA2Ab, IAb, but after excluding children with T1DM, such a correlation was no longer observed.

Conclusions:

1 There is a trend for ZnT8Ab to occur more frequently in children with autoimmune thyroid diseases- both Hashimoto’s thyroiditis and Graves’ disease compared to the healthy population

2 Presence of ZnT8Ab in patients with a AITD onset may be indicative of early stage of autoimmunity

3 Children with AITD and coexisting T1DM presented higher prevalence of ZnT8Ab compared to those with AITD without T1DM and the healthy population

4 Prevalence of ZnT8Ab in children with T1DM is not dependent on the coexistence of AITD

5 The prevalence of ZnT8Ab, GADAb, IA2Ab, IAb in children with AITD may indicate a potential association between thyroid autoimmunity and pancreatic islet autoimmunity

6 Children with AITD should be taken into consideration to islet autoantibody T1DM screening

7 Children with AITD show a higher risk of developing other autoimmune diseases, most commonly type 1 diabetes mellitus

8 Patients with Hashimoto's disease are more predisposed to developing an additional autoimmune disease than those with Graves’ disease