TYPES OF AUTOIMMUNE POLYENDOCRINOPATIES IN CHILDREN WITH DIABETES MELLITUS TYPE 1 AND THYROID DISEASE

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Abstract

Background&Aims: If are associated multiple autoimmune endocrine diseases, the term is polyglandular autoimmune syndrome (PAS). The main autoimmune polyendocrinopaties encountered are: PAS I (chronic mucocutaneous candidiasis, chronic autoimmune hypoparathyroidism, and autoimmune Addison disease), PAS II (autoimmune Addison's disease, autoimmune thyroid disease and/or type 1 diabetes) and PAS III (autoimmune thyroid disease + other autoimmune diseases). The purpose of this study is to determine the types of PAS in a group of children with thyroid diseases and diabetes mellitus (DM) type 1.

Methods: The studied group was of 83 children with DM type 1 (71 girls and 12 boys), aged between 7 and 17 years. The methods of investigation were represented by clinical, imaging, biochemical, hormonal and immunological parameters.

Results: The prevalence of PAS in the study group was: type I – 0%, type II - 6.02 % (80% girls and 20 % boys, $p = 0.057$, $X^2 = 3.6$) and type III - 59.03 % (85.71% girls and 14.29% boys, $p < 0.001$, $X^2 = 50$). We don't find significant differences between boys and girls in the case of PAS type II, but in type III prevailed girls, and the type was III a.

Conclusions: The most frequent PAS was type III, subtype III a, follow by type II. Because Addison's disease was asymptomatic, identifying through the presence of specified antibodies, if we have a children with two or more autoimmune disease, we must investigate him for another possible autoimmune disease.

Keywords: Diabetes mellitus type 1, thyroid disease, polyglandular autoimmune syndrome, children

Introduction

Type 1 diabetes is often associated with endocrine and systemic disease with autoimmune etiology as: Graves-Basedow disease, Hashimoto's thyroiditis, Addison's disease, celiac disease, pernicious anemia, myasthenia gravis, vitiligo, etc. (Cooper et al, 2003).

From people with type 1 diabetes, ≈ 1 in 100 patients will develop Graves' disease (De Block, 2000) and ≈ 1 in 20 patients are generally affected by hypothyroidism (De Block, 2000). The frequency of DM type 1 association with hyperthyroidism and hypothyroidism ranges from 3.2% to 4.6% and from 0.7% to 4% (Radaideh et al, 2003).

Addison's disease is extremely rare in the general population, affecting ≈ 1/ 200 patients in the population with type 1 diabetes (De Block, 2000).

Celiac disease occurs in 1/20 patients with type 1 diabetes, pernicious anemia in 1/50 patients with type 1 diabetes (De Block, 2000).

If are associated multiple autoimmune endocrine diseases, the term is polyglandular autoimmune syndrome (PAS). Polyglandular autoimmune syndromes comprise a group of autoimmune diseases characterized by inability of the endocrine glands to produce hormones. The endocrine abnormalities tend to occur together. It is estimated that about a quarter of patients with one gland hypofunction present another endocrine glands. It is therefore recommended that if is a one gland hypofunction to assess the function of other endocrine glands.

There are several classifications of autoimmune polyendocrinopaties, but was universally accepted Neufeld and Blizzard classification:

- **PAS I** - chronic mucocutaneous candidiasis, autoimmune chronic hypoparathyroidism, autoimmune Addison's disease
- **PAS II** - autoimmune Addison's disease, autoimmune thyroid disease and / or type 1 diabetes (Addison's disease should always be present)
- **PAS III** - autoimmune thyroid disease + another autoimmune diseases (excluding autoimmune Addison's disease, autoimmune chronic hypoparathyroidism, autoimmune chronic candidiasis)
- **PAS IV** - 2 or more autoimmune organo-specific diseases (not covered in the type I, II or III).

The most common are the PAS I, II and III.

Polyglandular autoimmune syndrome type I (PAS I) usually begins in childhood, mucocutaneous candidiasis being usually the first manifestation, usually followed by hypoparathyroidism and Addison's disease (Aldasouqi et al, 2006, Myhre et al, 2001). Type 1 diabetes occurs in less than 4% of affected children, but increased to 12% in adults.

PAS-I is a very rare disorder. The largest number of patients was reported in Finland, where the prevalence was estimated at 1/25,000 subjects. F/B ratio is 0.8 to 1.5/1; the 1998 statistical data show a ratio of 2.4 / 1.

PAS - I occur in children aged 3-5 years or in early adolescence, but always occur through the third decade of life. Mortality and morbidity of the syndrome seem to be equivalent to the individual components of the syndrome.

Type II polyglandular autoimmune syndrome (PAS II) (Betterle et al, 2004, Sivarajah et al, 2006) is the most common immunoendocrinopaty. It occurs in adulthood, affecting mostly women. Since most syndrome components have a long prodromal phase in which autoantibodies are present before developing the disease, current efforts are focused to establish whether immunosuppressive or immunomodulatory medication can prevent or stop the destructive processes.

In the U.S. about 14 to 20 per 1 million people are affected by this syndrome. The F/M ratio is 3 to 4/1. PAS - II appears in the third and fourth decade of life. To date, PAS - II mortality and morbidity has not been estimated from the clinical point of view, but it is considered as equal to the mortality and morbidity of the individual components.

Polyglandular autoimmune syndrome type III (PAS - III) (Aung et al, 2006) is associated with the following diseases: celiac disease, hypogonadism, myasthenia gravis, sarcoidosis, Sjogren's syndrome, rheumatoid arthritis, gastric cancer, malabsorption due to the pancreatic exocrine deficiency, and can be classified in three subcategories:

- PAS III a - autoimmune thyroiditis with DM type 1
- PAS III b - autoimmune thyroiditis with pernicious anemia
- PAS III c - autoimmune thyroiditis with vitiligo and / or alopecia and / or other autoimmune diseases

PAS III exact prevalence is unknown. PAS III is more common in women than in men. PAS III is typically observed in middle-aged women, but can occur in people of any age. Mortality and morbidity are determined by the individual components of the syndrome.

Another way of classifying autoimmune polyendocrinopathies and associated nonendocrine systemic immunopathies was proposed by Volpe:

- **Autoimmune endocrinopathies** – Graves-Basedow disease, Hashimoto's thyroiditis, idiopathic Addison's disease, insulin-dependent diabetes, autoimmune gonadal failure, autoimmune hypoparathyroidism, autoimmune pituitary disease, autoimmune infertility (sperm antibody)

- **Nonendocrine diseases** - pernicious anemia, vitiligo, myasthenia gravis, Sjogren's syndrome, rheumatoid arthritis, idiopathic thrombocytopenic purpura, chronic active hepatitis, primary biliary cirrhosis

DM is associated with endocrine disorders and in other autoimmune polyglandular syndromes (Dănciulescu, 2004), found with a much lower frequency.

Type B insulin resistance syndrome is caused by insulin antireceptor antibodies. One third of patients have associated autoimmune diseases as lupus erythematosus, autoimmune thyroid disease. Although blood glucose levels are elevated secondary to extreme insulin resistance, ketoacidosis is not characteristic. Patients may present spontaneous remissions and severe hypoglycemia (insulin-like side effects of insulin antireceptor antibodies, effects demonstrated in vitro).

POEMS syndrome include diabetes, primary gonadal failure, sensory and motor neuropathy, bone lesions, hyperpigmentation.

DIDMOAD syndrome is an autosomal recessive disorder which includes diabetes insipidus, diabetes mellitus, optic atrophy, deafness. DM is usually the first manifestation in children.

Trisomy 21 (Down syndrome) is relatively frequently associated with the presence of type 1 diabetes and thyroiditis. This suggests that chromosomal abnormalities influence the autoimmune processes or that susceptibility to develop autoimmune disorders may be associated chromosomal abnormalities.

METHODS OF INVESTIGATION
MATERIAL AND METHOD
INVESTIGATED POPULATION

The group of children was represented by 83 subjects aged between 7-17 years.

All children from the study group had Type 1 diabetes.

In the studied group, the gender distribution of the children was 5.9/1, represented by 71 girls (85.54%) and 12 boys (14.45%).

The methods of investigation were represented by **clinical data** - case history, current status, **imagistic**- thyroid ultrasound, **biochemical** - ***for glycemic balance***: fasting blood glucose, glycosylated hemoglobin, ***investigation of the thyroid gland***: TSH, FT_4, FT_3, thyroid antibodies, ***investigation of the adrenal gland:*** cortisol, 21-hydroxylase antibodies, ***gonadotropins:*** FSH, LH and appropriate sex hormones (testosterone, estradiol), ***investigation of celiac disease***: antitissue transglutaminase antibodies, ***investigation of pernicious anemia***: complete blood count with mean cell volume and vitamin B_{12} levels.

Determination of plasma glucose was performed by enzyme technique with glucosooxidasis. Normal values were taken between 70 - 110 mg%; diabetes mellitus - values equal or over 126 mg%, impaired glucose tolerance - values between 110 - 125 mg% and the OGTT at 2 h between 140 - 200 mg% and impaired fasting glucose - values between 110 - 125 mg% and OGTT at 2 h under 140 mg%.

Determination of HbA1c was achieved through the DiaStat for measuring HbA1c reported to the total HbA.

To determine ***the TSH level in plasma, the free fraction of triiodotironin (FT_3), and the plasma free fraction of thyroxin (FT_4)*** were performed a quantitative method ARCHITECT; witch is an immunological method, Chemilumnescent Microparticle Immunoassay (CMIA). Normal values were following: TSH = 0.465 - 4.68 Miu/ml, FT_3 = 3.69 -10.4 pmol/l, FT_4 = 10 - 28.2 pmol/l.

To obtain ***the level of cortisol*** was performed the technique IMMULITE / IMMULITE 1000, an imunometric method, in solid phase, competitive, of chemiluminescent, Immuno Chemilumino Enzymometric assay (ICEM). It was considered normal: a.m. 5 - 25 microgram/dl.

FSH level was measured quantitatively by the ARCHITECT method; a Chemilumnescent Microparticle Immunoassay. Reference values: determined with ARCHITECT test (Table I).

Table I. The reference values for FSH

Population field	mIU/ml
Women:	
- Follicular phase	3.35 – 21.63
- Ovulating phase	4.97 – 20.82
- Luteal phase	1.11 – 13.99
- Postmenopausal	2.58 – 150.53
Men	1.37 – 13.58

LH level was measured quantitatively by the ARCHITECT method; a Chemilumnescent Microparticle Immunoassay. Reference values: determined with ARCHITECT test (Table II).

Table II. The reference values for LH

Population field	mIU/ml
Women:	1.26 – 10.05
- Follicular phase	2.57 – 26.53
- Ovulating phase	18.06 – 90.23
- Luteal phase	0.67 – 23.75
- Postmenopausal	
Men	1.09 – 92.45

Testosterone was determinate by ELISA method. The references values are depending by age and gender:

Adults:
- men: 0.019 - 0.145 nmol/L;
- women in fertile period: < 0.014 nmol/L;
- pills: 0.001 - 0.0069 nmol/L;
- postmenopausal: 0.0003 - 0.0058 nmol/L.

Estradiol was determinate by immunochemical with electrochemiluminiscent detection method (ECLIA). The references values are depending by age and gender, and at women also with the menstrual cycle period and pregnancy (Table III).

Table III. The reference values for estradiol

Age and gender	***References values (pmol/L)***
Adults – ***Women*** • Follicular phase	46.0 - 607
• Ovulating phase	315 - 1828
• Luteal phase	161 - 774
• Postmenopausal	<18.4 - 201
– ***Men***	28.0 - 156
Pregnancy (first quarter)	789 – 15781
Children (1-10 years) • girls	22.0 - 99.1
• boys	<18.4 - 99.1

The immunological parameters were represented by autoimmune thyroid markers - antibodies (antiTPO and antiTg antibodies).

To determine ***serum levels of antiTPO antibodies*** it was used the kit AxSYM antiTPO, an immunological method (Microparticle Enzyme Immunoassay) (MEIA). Normal values: antiTPO antibodies <35 IU/ml.

To determine ***serum levels of antiTg antibodies*** it was used the kit AxSYM antiTg, a MEIA method as well (Microparticle Enzyme Immunoassay). Normal values: antiTg antibodies <55 IU/ml.

To determine ***21-hydroxylase (anti 21-OH antibodies) antibodies level*** it was used the radioimunodetermination method combined with a technique of imunoprecipitation, based on human 21-OH marked with I 125 reacting with the antibodies anti 21-OH from the samples test and forming immune complexes that precipitated with the solid-phase of protein A. Normal range: <1 IU/ml

Antitissue transglutaminase antibodies were determinate by ELISA method.

References values: IgA, IgG : <10 U/mL: negative; ≥10 U/mL: positive.

Vitamin B_{12} levels were determinate by immunochemical with electrochemiluminiscent detection method (ECLIA). References values: 191 - 663 pmol/L (for European population).

Determination of complete blood count was achieved with automatic method: electric impedance method. Normal values (for children): erythrocytes = 4 - 5.5 mil/mm^3, leucocytes = 4500 – 11000 mil/mm^3,

plateled = 150000 – 450 000/$mm^{3,}$ hematocrit (Ht): 32 – 44 %, hemoglobin (Hb): 9.5 – 15.5 g/dl.

Constants and red cell indices are calculated automatically, depending on the values of Hb, Ht and red blood cells (RBC) count. Normal values**:** mean corpuscular volume (MCV) = 80 - 100 fl, mean corpuscular hemoglobin concentration **(**MCHC) = 32 - 36 g Hb/100 ml erythrocytes, mean corpuscular hemoglobin (MCH) = 27 - 32 pg.

Thyroid ultrasound was performed in all cases and allowed us to measure thyroid volume, thyroid study and the changes in parenchyma's density.

An increased density, uniform, characterizes normal thyroid parenchyma easily distinguished from the neck muscles that are hypo dens.

Inflammatory processes and autoimmune pathology appears hypo dens**.** The scale was assessed as being discreet +, moderate ++ and marked +++.

In the autoimmune thyroid disease the parenchyma of the gland appears hypo dens.

Chronic autoimmune thyroid disorder appears with a hypoecogenity of the parenchyma and normal or increased thyroid volume.

STATISTICAL ANALYSIS

For statistical analysis we used Microsoft Excel and POP Tools from Microsoft Office 2003 and EPI 2000 program. To measure the quantitative variables were determined average (A) and standard deviation (SD), and to assess the gender differences we used the unpaired t test and ANOVA test, considering statistically significant a $p < 0.05$.

RESULTS AND DISCUSSION

In the case of children group, the main autoimmune endocrine associations were the following (Tab.IV).

Table IV. The prevalence of autoimmune endocrine diseases in children and adolescents group with type 1 diabetes

Asoociated autoimmune endocrine diseases	n	%
ACT	49	59.03%
Graves-Basedow disease	4	4.82%
ACT + asymptomatic Addison disease	5	6.02%

From the 83 cases of children and adolescents, in 53 cases were associated with two autoimmune endocrine disorders, and in 5 cases three autoimmune endocrine disorders. Type 1 diabetes was present in all 58 cases. In 54 cases with type 1 diabetes was associated ACT, and in 4 cases

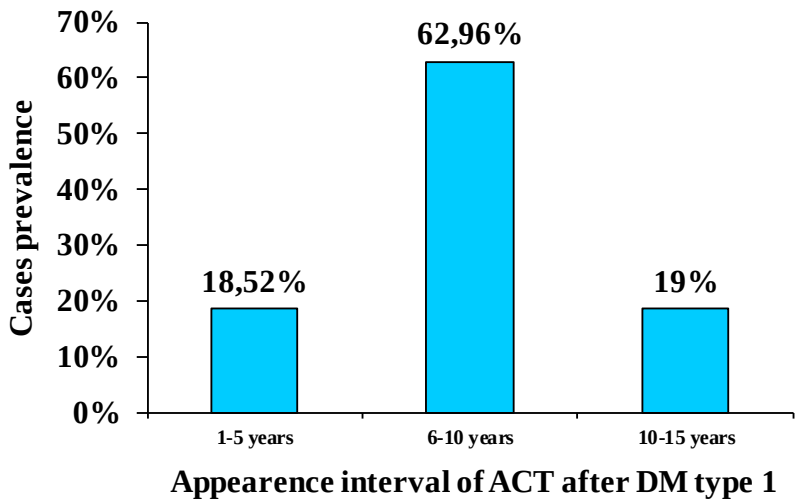
Graves-Basedow disease. In 5 cases with type 1 diabetes and ACT was associated asymptomatic Addison's disease.

Asymptomatic Addison's disease was diagnosed in 5 children by dosing 21-OH antibodies in 37 children from the study. From the 5 patients with positive titers, 4 were females and 1 male. The titers were between 1 to 2 IU/ml in 4 patients, only 1 patient presented titers of 2-3 IU/ml.

In the children and adolescents group of, in all cases of diabetes type 1/ACT association, the first immunopathy was DM type 1, followed by ACT appearance at a variable period of time (10 cases after 10-15 years, 34 cases after 6 – 10 years and 10 cases after 1-5 years of development of DM type 1) (Figure 1). In the case of association DM type 1/Graves-Basedow disease, the latter appeared before type 1 diabetes with 1 year in all 4 cases.

We don't have symptomatic cases of Addison's disease in children group, but only 5 cases of asymptomatic Addison's disease identifying through the presence of specified antibodies.

Figure 1. Appearence interval of ACT after DM type 1



The main types of endocrine organo-specific immunopathies diagnosed in the studied group of children and adolescents in are shown in Tab.V.

Table V. Organo-specific endocrine immunopathies diagnosed in children and adolescents group

Endocrine disorder type	Children group		Female		Male	
	n	%	n	%	n	%
DM type 1	58	69.88	47	81.03	11	18.96
ACT	54	65.06	47	87.03	7	12.97
Graves-Basedow disease	4	4.82	4	100	-	-
Asymptomatic Adisson dissease	5	6.02	4	4.82	1	1.2

We don't find the presence of **PAS I** in the study group. PAS I called also Blizzard syndrome is characterized by immune deficiency in the defense against candida albicans, autoimmune endocrinopathies, and optionally ectodermal dystrophy (nail, hair, tooth enamel, cornea) (Dankle, 2006). It is transmitted autosomal recessive.

Initially, chronic mucocutaneous candidiasis occurs, rebellious to treatment, followed after several years by development of hypoparathyroidism and then by DM type 1 (Bal et al, 2002).

In the case of association between hypoparathyroidism and type 1 diabetes, the onset of type 1 diabetes are reported under the age of 10 years, being rare after 20 years (Zosin, 1997).

PAS II was present in 5 cases. Initially PAS II covered PAS II a (Smith syndrome) (Addison's disease associated with autoimmune thyroiditis) and PAS II b(Carpenter syndrome) (Addison's disease associated with type 1 diabetes). It was later revealed the possibility of a triple association: adrenal insufficiency, chronic autoimmune thyroiditis and type 1 diabetes.

In the case of PAS II onset is usually with adrenal insufficiency (ICSR), in time associating DM type 1; there are cases in which the diagnosis is performed simultaneously for ICSR and type 1 diabetes or type 1 diabetes diagnosis can precede their ICSR (Zosin, 1997).

The prevalence of PAS type II in the study group was 6.02 % (80% girls and 20 % boys, $p = 0.057$, $X^2 = 3.6$). In United States, approximately 14 – 20 people per million populations are affected by polyglandular autoimmune syndrome type II. Observations have revealed, however, that the disease is much more prevalent if sub clinical forms are included (Bizarro, 2008).

Regarding the gender, we don't find significance difference between boys and girls, but in United States the female – to – male ratio of polyglandular autoimmune syndrome type II is 3 – 4:1 (Bizarro, 2008). Normally, the polyglandular autoimmune syndrome type II occurs in the third or fourth decade of life.

In all cases was a triple association: adrenal insufficiency, chronic autoimmune thyroiditis and type 1 diabetes. We don't have symptomatic cases of Addison's disease in children group, but only 5 cases of asymptomatic Addison's disease identifying through the presence of specified antibodies.

The most frequently detected PAS was **PAS III** containing after Blizzard's and Neufeld classification, association of autoimmune thyroid disease (Hashimoto's chronic thyroiditis, idiopathic myxedema, asymptomatic autoimmune thyroiditis, Graves' disease, endocrine ophthalmopathy) with:

- one or more autoimmune diseases - diabetes type 1 - type 3a
- association with gastric atrophy, pernicious anemia - type 3b
- association with vitiligo, alopecia, myasthenia gravis - type 3c.

In the case of PAS III, its worldwide prevalence is not known. It turned out that occurs more frequently in women than in men and at middle age but can occur at any age. The most common PAS III is the type 3a (DM type 1 association with chronic autoimmune thyroiditis). DM type 1 can be consecutive or may precede thyroiditis (Zosin, 1997).

The prevalence of PAS type III in the study group was 59.03 % (85.71% girls and 14.29% boys, $p < 0.001$, $X^2 = 50$), and the type was PAS III a (DM type 1 + autoimmune chronic thyroiditis). The medium age was 14.46 ± 2.4 years.

In the study group was a net predominance of females.

To endocrine immunopathies may be associated with a variable incidence no endocrine organo-specific systemic diseases.

In our studied group, the patients presented no endocrine organo-specific autoimmune diseases. They were represented by vitiligo met at 1.2 % cases and by the decalvant pelada found in 1.2 % of cases. In 1 case was associated vitiligo, characterized by skin pigmentation due to autoimmune destruction of melanocytes. It occurred before the onset of endocrine immunopathies. Decalvant pelada was met in 1 case and also precede the onset of autoimmune endocrinopathies.

The main endocrine and no endocrine immune association in children study group are shown in Fig.2.

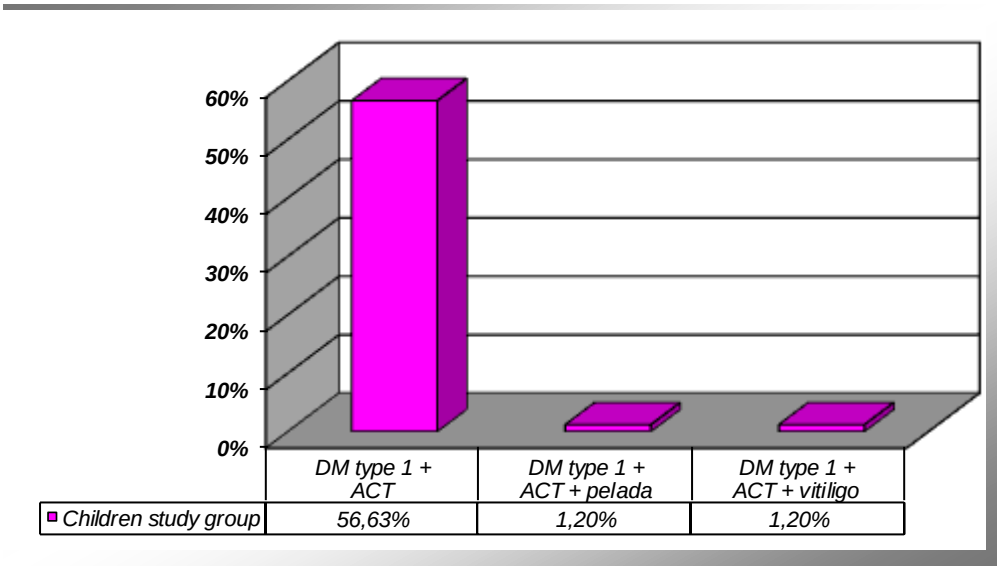


Figure 2. Endocrine and nonendocrine immune association in the group of children and adolescents with type 1 diabetes

The medium interval between onset age of type 1 diabetes and the occurrence of ACT was 8.59 ± 3.24 years.

No case presented the first endocrine immunopathy ACT, followed by type 1 diabetes.

In 4 cases (4.82%) we found the initial presence of Graves-Basedow disease, followed by an interval of 1 year by the occurrence of DM type 1. All the subjects were girls, with the medium age 16.75 ± 0.5 years.

Conclusion

We don't find PAS I in our study group.

In the children group, PAS type II comprises 3 diseases: adrenal insufficiency, chronic autoimmune thyroiditis and type 1 diabetes; we don't find significance difference between girls and boys**.** Because Addison's disease was asymptomatic, identifying through the presence of specified antibodies, if we have a children with two or more autoimmune disease, we must investigate him for another possible autoimmune disease.

The most frequent PAS was PAS III, subtype PAS III a (DM type 1 + autoimmune chronic thyroiditis), which prevailed in females.

So, if a child have an autoimmune disease, we must investigate him to another autoimmune diseases through determination of specific antibodies (frequent this autoimmune diseases are asymptomatic, identify through the presence of autoantibodies).

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