

PREVALENCE OF POLYGLANDULAR AUTOIMMUNE SYNDROME TYPE III IN A GROUP OF ADULTS WITH THYROID DISEASES AND DIABETES MELLITUS

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Abstract:

Polyglandular autoimmune syndrome (PAS) is made up of a group of autoimmune disorders of the endocrine glands. Polyglandular autoimmune syndrome type III (PAS III) comprises autoimmune thyroiditis, immune-mediated diabetes mellitus, celiac disease, hypogonadism, myasthenia gravis, sarcoidosis, Sjogren syndrome, rheumatoid arthritis, gastric neoplasia, and malabsorption. The purpose of this study is to determine the prevalence of PAS type III in a group of adults with thyroid diseases and diabetes mellitus (DM). The studied group was of 350 cases with an age between 17-79 years.

The group of adults was subdivided according to the type of changes in the glycemic balance in 2 subgroups: DM type 1 represented by 60 cases (17.14%) and DM type 2 represented by 290 cases (82.86%).

The methods of investigation were represented by clinical, imaging, biochemical, hormonal and immunological parameters.

Key Words: Diabetes Mellitus, Autoimmune Thyroid Disease, Polyglandular Autoimmune Syndromes, Adults

Introduction

Polyglandular autoimmune syndrome (PAS) is made up of a group of autoimmune disorders of the endocrine glands [10]. There are 3 autoimmune polyglandular syndromes:

Polyglandular autoimmune syndrome type I (PAS I) is an autosomal recessive disorder caused by a mutation in the short arm of chromosome 21, characterized by the triad: muco-cutaneous candidiasis, hypoparathyroidism and Addison's disease.

The symptoms and signs appear in childhood; candidiasis is usually the first sign, followed usually by hypoparathyroidism and Addison disease [1, 12]. DM type 1 occurs in less than 4% of affected children, but increases to 12% by adults.

Polyglandular autoimmune syndrome type II (PAS II) [3, 13] is the most common endocrinopathy. Occurs in adult life and affects mostly women. The same patient has two or more of the following conditions: Addison's disease, Graves's disease, autoimmune thyroiditis, DM type 1, primary hypogonadism, myasthenia gravis and celiac disease. Most disorders are associated with the following HLA: A1, B8, DR3 (DQA1 * 0501, DQB1 * 0201) and DR4 (DQA1 * 0301, DQB1 * 0302). The autoimmune syndrome disorders present usually a long prodromal phase and the antibodies are present prior to the development of the disorder.

Polyglandular autoimmune syndrome type III (PAS III) [2] is a **PAS II** syndrome, but without the adrenocortical involvement. It comprises a group of autoimmune disorders characterized by severe glandular insufficiency. A quarter of the patients with hypo functional glands present other endocrine diseases as well. This syndrome is associated with diseases as: organ-specific autoimmune diseases (celiac disease, hypogonadism, and myasthenia gravis), organ-nonspecific or systemic autoimmune diseases (sarcoidosis, Sjogren syndrome, and rheumatoid arthritis), other diseases (gastric carcinoid tumor, malabsorption due to exocrine pancreatic deficiency), and may be classified into the following 3 subcategories:

- PAS III A – Autoimmune thyroiditis with immune-mediated diabetes mellitus
- PAS III B - Autoimmune thyroiditis with pernicious anemia

- PAS III C - Autoimmune thyroiditis with vitiligo and/or alopecia and/or other organ-specific autoimmune disease

Autoimmunity, environmental factors, and genetic factors are the 3 major factors that should be considered in the physiopathology of PAS III.

Autoimmune disease affecting a single endocrine gland is frequently followed by impairment of other glands, resulting in multiple endocrine failures. The identification of circulating organ-specific auto antibodies provided the earliest and strongest evidence for the autoimmune pathogenesis of polyglandular failure syndromes [2].

Some studies show that environmental precipitators of autoimmunity might play a role in polyglandular autoimmunity. Viral infection may exaggerate the ongoing immune response and precipitate glandular failure (ex. the role of congenital rubella infection in etiopathogenesis of type 1 diabetes mellitus and hypothyroidism) [10].

PAS III, as well as PAS II, is associated with HLA class II genes with apparently distinctive HLA alleles for each. The underlying non-HLA genes of PAS III remain to be further defined genetically. PAS III is often observed in individuals in the same family, suggesting that its inheritance could be an autosomal dominant trait with incomplete penetrance. [6, 9, 15].

Multigenetic involvement in the development of the individual components of PAS III has been proved. For example, DM type 1 is linked to several loci in non-HLA genomic regions. Furthermore, autoimmune thyroiditis also is polygenic [4].

Family and population studies showed that the PAS III A has a strong genetic background. Several gene variations present in both autoimmune thyroiditis and DM type 1 have been identified by whole genome and candidate gene approaches [4].

About PAS III epidemiology, the exact international prevalence is unknown. The morbidity and mortality of PAS III is determined by the individual components of the syndrome. PAS III typically is observed in middle-aged women but can occur in persons of any age; it is more common in females than in males and no racial or ethnic difference in its frequency has been reported [2].

Characteristic for PAS III is the absence of adrenal insufficiency. Once adrenocortical insufficiency develops, such patients are reclassified as having PAS II. The involvement of multiple glands may be apparent at the time of initial presentation, but, more commonly, individual glandular failure develops sequentially. No specific sequence exists by which the individual glandular failures develop. The clinical symptoms of PAS III are a constellation of manifestations of endocrine gland failures that comprise the syndrome [10].

Material And Method

Method

Investigated Population

350 people with DM (307 F and 43 M), aged between 18 and 79 years represented the studied group.

Depending on glyceimic balance, the group was divided into:

- the group with DM type 1 – 60 (17, 14%) (55 F and 5 M)
- the group with DM type 2 – 290 (82, 86%) (252 F and 38 M)

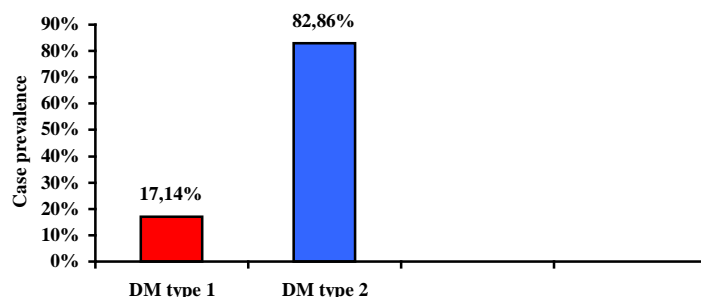


Fig. 1. Cases classification depending on glyceimic balance

Methods Of Investigation

The methods of investigation were represented by clinical data - case history, current status, imagistic- thyroid ultrasound, biochemical - for glyceimic balance: fasting blood glucose, glycosylated

hemoglobin, investigation of the thyroid gland: TSH, FT₄, FT₃, thyroid antibodies, investigation of the adrenal gland: ACTH, 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 B₁₂ levels, parietal cell and anti-intrinsic factor antibodies.

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 (FT₃), and the plasma free fraction of thyroxin (FT₄) 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, FT₃ = 3.69 -10.4 pmol/l, FT₄ = 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. The reference values for FSH

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

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 II. 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 radioimmunodetermination method combined with a technique of immunoprecipitation, 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

ACTH was determinate by immunoassay with chemiluminescent detection method.

Antitissue transglutaminase antibodies were determinate by ELISA method.

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

Vitamin B₁₂ levels were determinate by immunochemical with electrochemiluminiscent detection method (ECLIA). References values: 191-663 pmol/L (for european population).

Parietal cell antibodies were determinate by indirect immunofluorescence. References values: negative.

Anti-intrinsic factor antibodies were determinate by ELISA method.

References values: < 6 U/mL: negative.

Determination of complete blood count was achieved with automatic method: electric impedance method. Normal values: erythrocytes = 4-5.5 mil/mm³ (men: 4.9 ± 0.7 mil/mm³, women: 4.3 ± 0.6 mil/mm³), leucocytes = 5000 – 9000 mil/mm³, plateled = 150000 – 350 000/mm³, hematocrit (Ht): men 45 ± 7%, women 42 ± 5%, hemoglobin (Hb): men: 15 ± 2 g/dl, women: 14 ± 2 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 hypoeogeneity of the parenchyma and normal or increased thyroid volume.

Results And Discussion

In the group of adults 17.14% had DM type 1 and 82.86% had DM type 2.

In the group with DM type 1 the main endocrine immune combinations were represented by DM type 1 with autoimmune chronic thyroiditis (ACT). Other endocrine immune associations were represented by autoimmune ovarian insufficiency and the nonendocrine disorders as vitiligo, alopecia, Biermer anemia (Tab. III).

Table III. Prevalence of endocrine autoimmune disorders in the studied group

Associations	Subject group	
	No.	%
DM type 1	60	
DM type 1 + ACT	31	51.66%
DM type 1 + ACT + decalvant pelad	2	3.33%
DM type 1 + ACT + vitiligo	3	5%
DM type 1 + ACT + autoimmune ovarian failure	6	10%
DM type 1 + ACT + vitiligo + Biermer anemia	1	1.66%
DM type 2	290	
DM type 2 + ACT + vitiligo	6	2.06%

In the group of adults with DM type 1 the first immunopathy was DM type 1, present in 24 of the cases and was associated with ACT in all 24 cases. In 2 cases, thyroid disorder and DM type 1 were detected at the same time. In 17 cases thyroid disorder preceded the DM type 1 (Tab. IV).

Tracking the association with autoimmune ovarian insufficiency led to determine the levels of FSH, which was increased > 25 IU/l in 6 cases. Primary ovarian insufficiency (early menopause) usually occurs before the age of 40 years (in the absence of iatrogenic causes) and its clinical signs are secondary amenorrhea and hypergonadotropism with hypoestrogenemia.

Autoimmune ovarian insufficiency (AOI) is usually associated with other autoimmune pathology such as diabetes mellitus type 1, Addison's disease, ACT, vitiligo, etc.; its diagnosis is difficult and it is usually based on the exclusion of other possible causes of primary ovarian insufficiency and the notice of autoimmune etiology [2].

Also estrogen therapy in autoimmune ovarian insufficiency may increase the risk of cardiovascular disease [11].

Endocrine immunopathys may be linked to a variable incidence of systemic organ-specific nonendocrine disorders.

In 4 cases it was associated vitiligo, which occurred before the onset of endocrine immunopathys. Pelade decalvant appeared in 2 cases and also preceded the onset of autoimmune endocrinopathys. One case in the group of adults with autoimmune endocrine diseases had associated more than one nonendocrine autoimmune disorder respectively Biermer anemia and vitiligo.

Table IV. Range (years) between the onsets of immunopathys in adults with type 1 diabetes

Period of time	No. of cases	Media \pm SD (age)
Onset DM – Onset ACT	24	22.29 \pm 12.42
Onset ACT - Onset DM	17	2.47 \pm 1.94

In adults, the average time between the onset of type 1 DM and ACT was 22.29 ± 12.42 years.

In 17 cases the first immunopathy was ACT, followed at a distance of 2.47 ± 1.94 years by DM type 1.

All the patients with DM type 1 and ACT were women, with the median age 43 ± 18.95 years and the average age of onset 30.46 ± 22.94 years. It was no thyroid familial history disease found.

In the study group, all patients had DM type 1 clinically manifest, all being treated with insulin in different therapeutic schemes

Prevalence of ACT in DM type 2 was 26.55% (77 patients, 69 F and 8 M). In adults with DM type 2 PAS type III was found in 6 (2.06%) cases, of which all have ACT + vitiligo. The first disease was vitiligo follow by ACT after 2-5 years. All cases were females. The median age was 55 ± 14.14 years and the average age of onset was 53.5 ± 12.02 years. DM type 2 appears after ACT, at 5-10 years, possible because of replacement therapy for thyroid disease. It was no thyroid familial history disease found. At all patients with DM type 2 the treatment was diet.

Also in these cases it is useful to determinate the antibodies for diabetes because these patients may be latent autoimmune diabetes in adults (LADA) types and in time requires insulin.

The prevalence of PAS III is unknown. It is more often met in women than by men; usually by middle-aged women but it can occur in people of any age. Death is determined by the individual components of the syndrome [2].

So, in our study, the prevalence of PAS type III was 14% (100% F and 0% M, $p < 0.001$, $X^2 = 52.69$).

PAS type III prevalence in DM type 1 was 71.66% (100% F and 0% M, $p < 0.001$, $X^2 = 67.01$) and 2.06% in DM type 2 (100% F and 0% M, $p = 0.01$, $X^2 = 6.06$).

In the case of DM type 1 we have PAS type III A, and in the case of DM type 2 only PAS type III C.

Significant differences regarding PAS prevalence were found between the group with DM type 1 and 2 (71.66% vs. 2.06%, $p < 0.001$, $X^2 = 200.01$).

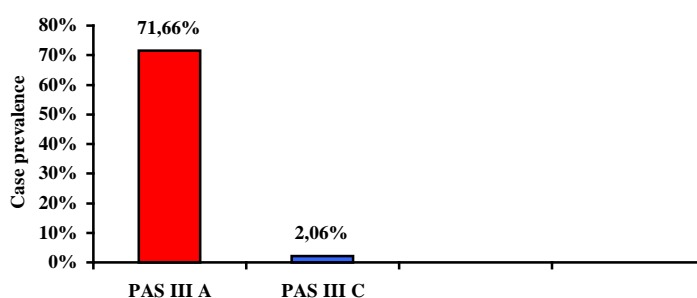


Fig. 2. PAS prevalence in study group

In general, in the first stage of PAS antibodies levels are elevated. In the second stage the disease is sub clinical and in the third stage becomes clinically manifested.

By ACT, 24 cases were with euthyroidism and 96 cases with hypothyroidism (76 clinical cases and 20 cases sub clinical). 24 cases did not require treatment; the remaining had substitution treatment with thyroid hormones. AntiTPO antibodies were present by 27 of cases of ACT and DM type 1, the remaining cases presenting insignificant values.

Ideal is to determine the presence of antibodies, especially in DM type 1, because they may be present by subjects without clinical symptoms. If their levels are raised, it is good to monitor annual the TSH level and if it is normal it is recommended to doze antithyroid antibodies by intervals of 2-3 years [5, 14].

Also, if the disease is autoimmune, the patient should be investigated for other autoimmune associations of endocrine or nonendocrine nature.

A study in Czech Republic on 51 patients with DM type 1 showed that it is associated with autoimmune thyroid diseases, with Addison's disease and celiac disease.

The authors recommend finding the specific antibodies for each disease, to diagnose the disease in the initial phase, and to prevent the complications that will affect the quality of the patients' life [7].

If DM type 2 is present it is recommended to evaluate TSH levels, and if it is normal, to repeat this evaluation every 5 years.

If pre-existing thyroid pathology is present it is recommended to evaluate plasma glucose levels annually.

Conclusion

The prevalence of PAS type III in the study group was 14%; all the patients with this were middle-aged women.

PAS type III has prevailed in the group with type 1 diabetes (71.66%) due to autoimmune origin, part of the polyglandular autoimmune syndrome (PAS) type III A.

In the case of DM type 2 the prevalence of PAS III was only 2.06%, and the type was PAS III C. In these cases the patients with autoimmune disease may be type latent autoimmune diabetes in

adults (LADA). So, if we have a patient with two or more autoimmune disease, we must investigate this for another possible autoimmune disease.

Many disorders involved in PAS present a long prodromal phase, characterized by the presence of characteristic antibodies for each disorder in part, before the clinical manifestations.

The treatment of patients with PAS involves early identification of all components.

The treatment of PAS is currently the treatment of each component of endocrine disorder (usually through hormone substitution therapy).

Isohormonal therapy has "immunomodulatory" capacities (hormone produced by the target organs may be able to influence autoimmunity).

Associations of specific autoimmune endocrinopathys require specific management. Controversial discussions are described in the literature on the effectiveness of thyroxin in patients with positive antibodies, but with euthyroidism or sub clinical hypothyroidism [8].

Some show a significant reduction of the TSH and of the anti-TPO antibodies in patients with autoimmune thyroiditis and euthyroidism after 1 year of treatment with thyroxin [8].

The PAS classification is not final. This may change over time, with the onset of new endocrine disorders or associations with new autoimmune determination.

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