# EPIDEMIOLOGICAL ASPECTS OF POLYGLANDULAR AUTOIMMUNE SYNDROME TYPE II IN A GROUP OF ADULTS WITH THYROID DISEASES AND DIABETES MELLITUS

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**Abstract**

**Background&Aims:** Polyglandular autoimmune syndrome (PAS) type II is the most common of the immunoendocrinopathy syndromes. It is characterized by the presence of autoimmune Addison disease in combination with thyroid autoimmune diseases, type 1 diabetes mellitus, primary hypogonadism, myasthenia gravis, and celiac disease. The purpose of this study is to determine the epidemiological aspects of PAS type II in a group of adults with thyroid diseases and diabetes mellitus (DM). **Methods:** The studied group was of 350 cases with an age between 18-79 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. **Results:** The prevalence of PAS type II in the study group was 3.14% (100% F and 0% M, $p<0.001$, $X^2=11.18$) and prevailed in middle – age women. PAS type II prevalence in DM type 1 was 11.66% and 1.38% in DM type 2 ($p<0.001$, $X^2=200.01$). **Conclusions:** PAS type II has prevailed in females and in the group with DM type 1 due to autoimmune origin.

**Keywords:** Diabetes mellitus, thyroid disease, polyglandular autoimmune syndrome type II, adults

## Introduction

Polyglandular autoimmune syndrome (PAS) is made up of a group of autoimmune disorders of the endocrine glands (Kahaly, 2009).

Polygandular autoimmune syndrome type II (PAS II) is the most common of the immunoendocrinopathy syndromes. It is characterized by the

obligatory occurrence of autoimmune Addison disease in combination with autoimmune chronic thyroiditis and/or type 1 diabetes mellitus. Primary hypogonadism, myasthenia gravis, and celiac disease also are commonly observed in this syndrome.

The definition of the syndrome depends on the fact that if one of the component disorders is present, an associated disorder occurs more commonly than in the general population. The most frequent clinical combination association is Addison disease and Hashimoto thyroiditis, while the least frequent clinical combination is Addison disease, Graves's disease, and type 1 diabetes mellitus.

The complete triglandular syndrome is sometimes referred to as Carpenter syndrome.

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 subclinical forms are included.

PAS II occurs primarily in adulthood, usually around the third and fourth decades of life. Middle-aged women have shown an increased prevalence of PAS II. The female-to-male ratio of polyglandular autoimmune syndrome type II is 3-4:1. To date, the mortality and morbidity rates of PAS II have not been clinically estimated. The mortality and morbidity of PAS II are believed to equal the mortality and morbidity of the individual component disorders. It is associated with HLA-DR3 and/or HLA-DR4 haplotypes, and the pattern of inheritance is autosomal dominant with variable expressivity (Obermayer-Straub et al, 1998).

The autoimmune syndrome disorders present usually a long prodromal phase and the antibodies are present prior to the development of the disorder.

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

The pathogenesis of polyglandular autoimmune syndrome type II is poorly understood (Baker, 1997, Betterle et al, 2004). The persons have some degrees of genetic susceptibility and than they are exposed to the autoimmune trigger, which could be an environmental or intrinsic factor (Ramos-Lopez et al, 2008). The trigger mimics the molecular structure of a self-antigen. An alternative explanation is that a breakdown in normal immunologic tolerogenesis occurs.

Next, a subclinical phase of active production of organ-specific autoantibodies occurs. This phase is followed by autoimmune activity in the respective organ, in which there is progressive glandular destruction. The individual is still asymptomatic.

Overt clinical disease subsequently develops when extensive organ damage, caused by the aforementioned autoimmune activity, has occurred. Evidence of this autoimmune phenomenon that may be responsible for this syndrome is based on whether the affected organs demonstrate a chronic inflammatory infiltrate composed of lymphocytes (mainly) (Obermayer-Straub et al, 1998).

Two other related autoimmune endocrinopathies exist, namely type I and type III.

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 (Myhre et al, 2001, Aldasouqi et al, 2006). DM type 1 occurs in less than 4% of affected children, but increases to 12% by adults.

Polyglandular autoimmune syndrome type III (PAS III) (Aung et al, 2006) is a PAS II syndrome, but without the adrenocortical involvement. It comprises a group of autoimmune disorders characterized by severe glandular insuffiency. 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

## 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 glycemic 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)

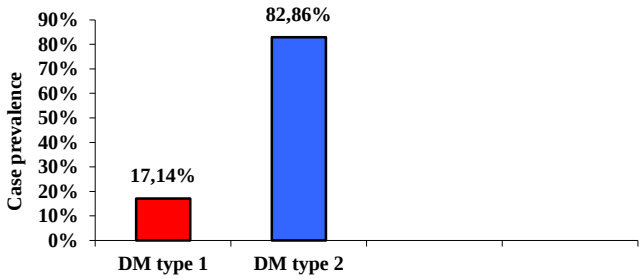


**Fig. 1**. Cases classification depending on glycemic balance

## Methods Of Investigation

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:*** 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 $B_{12}$ 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 ($FT_3$), 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.** 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.** The reference values for LH

| Population field | mIU/ml |
|---|---|
| **Women:** | |
| - Follicular phase | 1.26 – 10.05 |
| - Ovulating phase | 2.57 – 26.53 |
| - Luteal phase | 18.06 – 90.23 |
| - Postmenopausal | 0.67 – 23.75 |
| ***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.** 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

***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 $B_{12}$ 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/$mm^3$ (men: 4.9 ± 0.7 mil/$mm^3$, women: 4.3 ± 0.6 mil/$mm^3$), leucocytes = 5000 – 9000 mil/$mm^3$, plateled = 150000 – 350 000/$mm^3$, 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 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 group of adults 17.14% had DM type 1 and 82.86% had DM type 2**.**

The prevalence of PAS II in the study group was 3.14 % (11.66% in the case of DM type 1 and 1.38% in the case of DM type 2, $p < 0.001$, $X^2 = 200.01$). (Table IV).

**Table IV.** Prevalence of PAS II in the study group

| **Associations** | **Subject group** | |
|---|---|---|
| | **No.** | **%** |
| **DM type 1** | 60 | |
| **DM type 1 + ACT + asimtomatic Addison disease** | 7 | 11.66% |
| **DM type 2** | 290 | |
| **DM type 2 + ACT + Addison disease** | 4 | 1.38% |

In adults ***with type 1 diabetes mellitus***, the prevalence of PAS II was 11.66%. All were women, with a medium age 44.28 ± 23.57 years. We don't find family history of thyroid diseases or diabetes.

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

In 2 cases, the first disease was DM type 1; follow by ACT after 2 ± 0.5 years. In 5 cases, the first disease was ACT; follow by DM type 1 after 27.8 ± 23.13 years.

For appreciation association of Addison disease, were determinate plasma cortisol and 21-OH antibodies. The 21-OH values were significally higher in 7 cases, all theses were clinically asymptomatic.

In adults with ***type 2 diabetes mellitus***, the prevalence of PAS II was 1.38 %. All were women, with a medium age 61.75 ± 7.32 years, and the onset medium age for thyroid disease was 60.75 ± 8.38 years. We don't find family history of thyroid diseases or diabetes.

At all patients with DM type 2 the treatment was diet.

All the 4 cases with Addison diseases were symptomatic; the diagnosis was early, base on clinical symptoms. In the case of association between Addison disease and ACT, the first imunophathy was Addison disease; follow after 10 - 16 years by ACT.

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.

In the case of association of Addison disease, the substitution treatment may determinate unbalance of DM, especially of DM type 1 (Betterle et al, 2004).

Among patients with type 1 diabetes mellitus, thyroid autoimmunity and celiac disease coexist with sufficient frequency to justify screening. Measuring annual thyrotropin levels in individuals with type 1 diabetes mellitus is recommended as cost-effective.

Clinical history and examination suggesting evidence of more than 1 endocrine deficiency should prompt testing, to include serum autoantibody screening and an evaluation of end-organ function.

Serum auto antibodies screen – this helps to verify the autoimmune etiology of the disease and to identify persons who may later develop multi-endocrine deficiency. This test also is useful in screening asymptomatic family members who may develop autoimmune endocrine disease in the future.

Evaluation of end-organ function is necessary to confirm the diagnosis in patients with positive auto antibodies. Even if these antibodies are negative, still perform testing if clinical suspicion is high, because the sensitivity of these assays is not perfect. Some of these tests must be perform annually, because not all diseases manifest at the time of the initial diagnosis. (Sivarajah et al, 2006)

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. The substitution treatment with thyroxin may precipitate the adrenal insufficiency in the case of untreated Addison disease. Hypoglycemia or decrease of insulin requirement at patients with DM type 1 may signified the onset of the adrenal insufficiency. The asymptomatic forms of Addison disease must be treating with attention in acute stress condition. In Addison disease associate with ovarian insufficiency, the

substitution therapy with steroid hormones may prevent severe osteoporosis. Controversial discussions are described in the literature on the effectiveness of thyroxin in patients with positive antibodies, but with euthyroidism or sub clinical hypothyroidism (Hossein et al, 2005).

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 (Hossein et al, 2005).

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

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 (Eisenbarth et al, 2004, Tunbridge et al, 2000).

Also, if the disease is autoimmune, the patient should be investigated for other autoimmune associations of endocrine or no endocrine 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 (Gonem et al, 2007).

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.

The time course of the development of organ-specific autoimmunity makes it necessary to repeatedly reevaluate patients and their families over time. Provocative and suppressive testing frequently is necessary. (De Graaff et al, 2007, Förster et al, 1999).

**Conclusion**

The prevalence of PAS type II in the study group was 3.14%; all the patients with this were middle-aged women.

PAS type II has prevailed in the group with type 1 diabetes due to autoimmune origin.

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

So, if we have a patient with two or more autoimmune disease, we must investigate this and the asymptomatic family members for another possible autoimmune disease, using cost-effective tests.

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