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IgA and IgG Antigliadin, IgA Anti-tissue Transglutaminase and Antiendomysial Antibodies in Patients with Autoimmune Thyroid Diseases and Their Relationship to Thyroidal Replacement Therapy

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Summary

Celiac disease is a chronic illness of the small bowel caused by gliadin intolerance in genetically predisposed subjects. The aim of this study was to investigate serum levels of IgA and IgG antigliadin antibodies, IgA antiendomysial antibodies, and IgA anti-tissue transglutaminase antibodies in 169 patients with autoimmune thyroid diseases, i.e. chronic thyroiditis and Graves´ disease. Antiendomysial antibodies were positive in 2 out of 169 persons (1.18 %), IgA antigliadin antibodies in 15.98 %, IgG antigliadin antibodies in 51.48 %, and IgA anti-tissue transglutaminase in 14.79 %. The prevalence of positivity was higher compared to the 1312 control blood donors described in our previous study (Vančíková *et al.* 2002) ($p<0.05$). Patients with chronic thyroiditis treated with a high replacement dosage of levothyroxin (125-200 μg daily) had higher serum levels of IgA antigliadin antibodies in comparison with patients treated with a lower dosage (50-100 μg daily) (medians: 13.00 vs. 19.69, $p=0.033$). We found a negative correlation of IgA anti-tissue transglutaminase antibodies and total calcium serum levels ($r = –0.480$, $p=0.0236$, $n=22$). We can conclude that in persons with autoimmune thyropathy there is a high prevalence of positive antigliadin, anti-tissue transglutaminase and antiendomysial antibodies. Latent celiac disease may lead to impaired resorption of therapeutically administered levothyroxine, calcium, or other substances.

Key words

Celiac disease • Antigliadin antibodies • Thyroid autoimmunity

Introduction

Celiac disease is a chronic disease of the small bowel caused by gluten intolerance in genetically predisposed people with several features of autoimmunity (Tlaskalová *et al.* 2000): 1) HLA linkage (HLA-DQA1 0501, HLA-DQB1 0201), 2) associated autoimmune diseases as type 1 diabetes mellitus (De Block *et al.* 2001, Jaeger *et al.* 2001, Volta *et al.* 1997), autoimmune thyroid disease (Collin *et al.* 1994, Counsell *et al.* 1994, Sategna-Guidetti *et al.* 1998, Velluzzi *et al.* 1998, Cuoco *et al.* 1999, Valentino *et al.* 1999, Kowalska *et al.* 2000, Sategna-Guidetti *et al.* 2001, Volta *et al.* 2001), polyglandular autoimmune syndrome (Kaukinen *et al.*

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1999), systemic lupus erythematodes (Rensch *et al.* 2001), Sjogren´s syndrome (Tlaskalová-Hogenová *et al.* 1985), 3) intestinal lymphomononuclear infiltration, 4) presence of specific autoantibodies, 5) existence of defined autoantigens (autoantigen of endomysium defined as tissue transglutaminase, calreticulin), 6) response to corticosteroids and immunosuppressive treatment, 7) transfer of mucosal damage by intraepithelial lymphocytes, 8) transfer of the disease by lymphocytes during bone marrow transplantation, 9) stimulation of natural killer cell activity by gliadin in patients with celiac disease.

Celiac disease is considered to be a type of precancerosis (Holmes 1997, Marsh 1997, O´Boyle *et al.* 1998). Malignancy develops in 8-13 % of the patients with the illness (Tlaskalová *et al.* 2000), and the most common type is the non-Hodgin lymphoma from the CD8+ T cells EATCL (assuming a connection with an increase in antigen pressure after enhanced intestinal permeability). The incidence of small bowel, esophagus and pharynx carcinomas is even higher and there is debate about the potential influence of vitamin A deficiency (Logan *et al.* 1989). Another consequence is the possibility of celiac crisis accompanied by extreme diarrhea and metabolic disruption that can be fatal.

The determination of serum levels of IgA and IgG antigliadin antibodies (AGA-IgA and AGA-IgG), IgA antiendomysial antibodies (AEA), and IgA anti-tissue transglutaminase antibodies (ATA), plays an important role in the screening and diagnosis of celiac disease (Vitoria *et al.* 1999). The aim of this study was to find out the prevalence of these antibodies in people with autoimmune thyroid diseases (AITD) – chronic autoimmune thyroiditis (CT) and Graves' disease (GD), and to reveal their clinical significance. Likewise, it was of interest to ascertain whether their positivity has any relationship to thyroid function, more particularly to the dosage of levothyroxine replacement therapy in cases with subclinical or overt hypothyroidism.

Methods

Altogether, we examined 169 people between the ages of 16 and 82 years; the average age was 52 years. There were 153 women and 16 men, all with AITD proven clinically, through laboratory tests and sonography, and positive antibodies to thyroglobulin (TGB-Ab), and/or antibodies to thyroid peroxidase (TPO-Ab): 134 persons had chronic thyroiditis (25 untreated), 109 were treated with levothyroxin, and 35 patients had Graves' disease (8 with symptoms of thyrotoxicosis and 27 who were euthyroid during carbimazol treatment or after total thyroidectomy).

IgA and IgG antigliadin antibodies (AGA-IgA and AGA-IgG) were determined by the ELISA method that employs purified α-gliadin as an antigen. The results were related to the laboratory standard and were expressed as an index (normal values <30), the sensitivity is 65-95 % for AGA-IgA, 80-95 % for AGA-IgG, and the specificity 84-100 % for AGA-IgA, 70-95 % for AGA-IgG. IgA antiendomysial antibodies were determined by the immunofluorescent method using a preparation of primate GIT smooth muscle from IMMCO (USA) (evaluation: positive/negative, sensitivity 97-100 %, specificity 90-100 %). IgA anti-tissue transglutaminase antibodies (ATA) were determined by the ELISA method employing the Genesis package (reference values 1-10 $U.l^{-1}$, sensitivity as almost 100 %, specificity approximately 97 %). TGB-Ab and TPO-Ab, the thyroid-stimulating hormone (TSH) and free thyroxin (fT4) were determined by the RIA method (reference values for TGB-Ab, 1-60 $kIU.l.10^{-1}$; for TPO-Ab, 1-60 $kIU.l.10^{-1}$; for fT4, 9.8-23.1 $pmol.l.10^{-1}$; for TSH, 0.05-5.0 $mIU.l.10^{-1}$). Serum levels of overall calcium (Ca), lipids and C-reactive protein (CRP) were determined by the usual laboratory procedures in 22 patients. There was no case with a diagnosis of IgA deficit.

The results were compared with a random reference group of 1312 selected sera of voluntary blood-donors examined in our previous study (Vančíková *et al.* 2002) and were statistically evaluated by the χ^2-test, the Fisher exact test, the t-test, the Mann-Whitney test, the Pearson correlation coefficient, and the Spearman rank order correlation coefficient employing the Sigmastat program (Jandel Scientific, USA).

Results

The average TSH and fT4 serum concentration and the medians and prevalence of positivity of TPO-Ab, TGB-Ab, AGA-IgA, AGA-IgG, ATA and AEA in CT and GD groups are illustrated in Table 1.

Antiendomysial antibodies were positive in 2 out of 169 (1.18 %) persons with AITD (in patients with CT), compared to 0.45 % in the control subjects ($p<0.05$). AGA-IgA were positive in 15.98 % of cases with AITD, compared to 7.70 % in the control subjects ($p=0.002$), AGA-IgG in 51.48 % of cases with AITD, compared to

Table 1. Antigliadin, anti-tissue transglutaminase and antiendomysial antibodies in patients with thyroid autoimmune diseases.

		Grave´s disease	Chronic autoimmune thyroiditis	p
Number of patients		35	134	
AGA-IgA	median	14.500	13.140	0.578 *
	% of positivity	17.143	15.672	NS **
AGA-IgG	median	41.500	29.000	0.044 *
	% of positivity	65.714	47.761	NS **
ATA	median	1.455	2.000	0.430 *
	% of positivity	17.143	14.179	NS **
AEA	% of positivity	0.000	1.490	NS **
TGB-Ab	median	139.000	323.550	0.014 *
	% of positivity	100.000	100.000	NS **
TPO-Ab	median	815.500	734.000	0.410 *
	% of positivity	100.000	100.000	NS **
TSH	mean ± SD	2.6±3.1	6.1±13.9	0.168 ***
freeT4	mean ± SD	28.6±26.2	16.8±5.9	0.004 ***

*AGA-IgA, AGA-IgG: serum levels of IgG and IgA antigliadin antibodies (results were related to the laboratory standard and expressed as an index), ATA: serum levels of IgA anti-tissue transglutaminase antibodies(U.l.10^{-1}), AEA (positive/negative): IgA antiendomysial antibodies, TGB-Ab: serum levels of antibodies to thyroglobulin (U.l.10^{-1}), TPO-Ab: serum levels of antibodies to thyroid peroxidase (U.l.10^{-1}), TSH: serum levels of thyroid stimulating hormone (mIU.l.10^{-1}), freeT4: serum levels of free thyroxin (pmol.l.10^{-1}), p: level of significance, SD: standard deviations, * Mann-Whitney test, ** Fisher exact test, *** T-test, NS: not significant.*

7.00 % in the control subjects (p<0.001), and ATA in 14.79 % of cases with AITD, compared to 7.00 % in the control subjects (p=0.002) (Fig. 1).

The prevalence of positivity of AGA-IgA, AGA-IgG and ATA did not significantly differ in the CT and GD groups (AGA-IgA: 15.67 % vs. 17.14 %, AGA-IgG: 47.76 % vs. 65.71 %, ATA: 14.18 % vs. 17.14 %). Similarly neither group diverged significantly in their medians of serum concentrations of AGA-IgA (CT: 13.14 vs. GD: 14.50 U.l^{-1}, p=0.578) and ATA (CT: 2.00 vs. GD: 1.46 U.l^{-1}, p=0.430) (Fig. 2).

Median serum concentrations of AGA-IgG were significantly higher in the GD group, compared to the CT group (41.50 vs. 29.00 U.l^{-1}, p=0.044) (Fig. 2). Median values of AGA-IgA serum levels were higher in men, compared to women (20.13 vs. 13.05 U.l^{-1}, p=0.038), although it should be noted that fewer men than women were examined (16:153).

Positivity of at least one of the antibodies with the exception AEA (AGA-IgA or AGA-IgG or ATA) was found in 60.95 % patients with AITD (58.21 % CT and

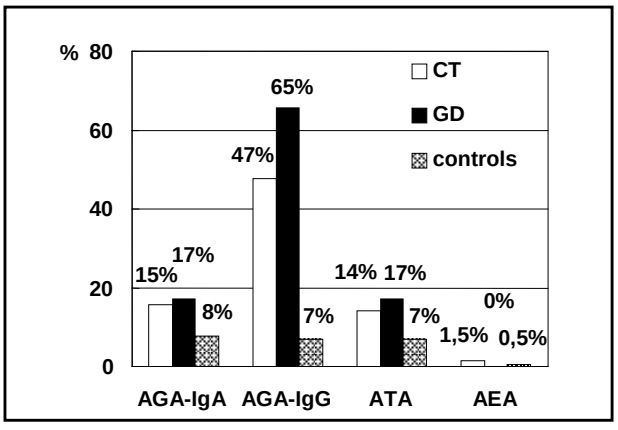

Fig. 1. *Prevalence of positive antigliadin, anti-tissue transglutaminase and antiendomysial antibodies in patients with autoimmune thyroid diseases. GD: Graves´ disease (hyperthyroid or euthyroid during carbimazol therapy or after total thyroidectomy), CT: chronic autoimmune thyroiditis (euthyroid or hypothyroid), controls: blood donors, AGA-IgA, AGA-IgG: positive serum levels of IgA and IgG antigliadin antibodies, ATA: positive serum levels of IgA anti-tissue transglutaminase antibodies, AEA: positive serum levels of IgA antiendomysial antibodies.*

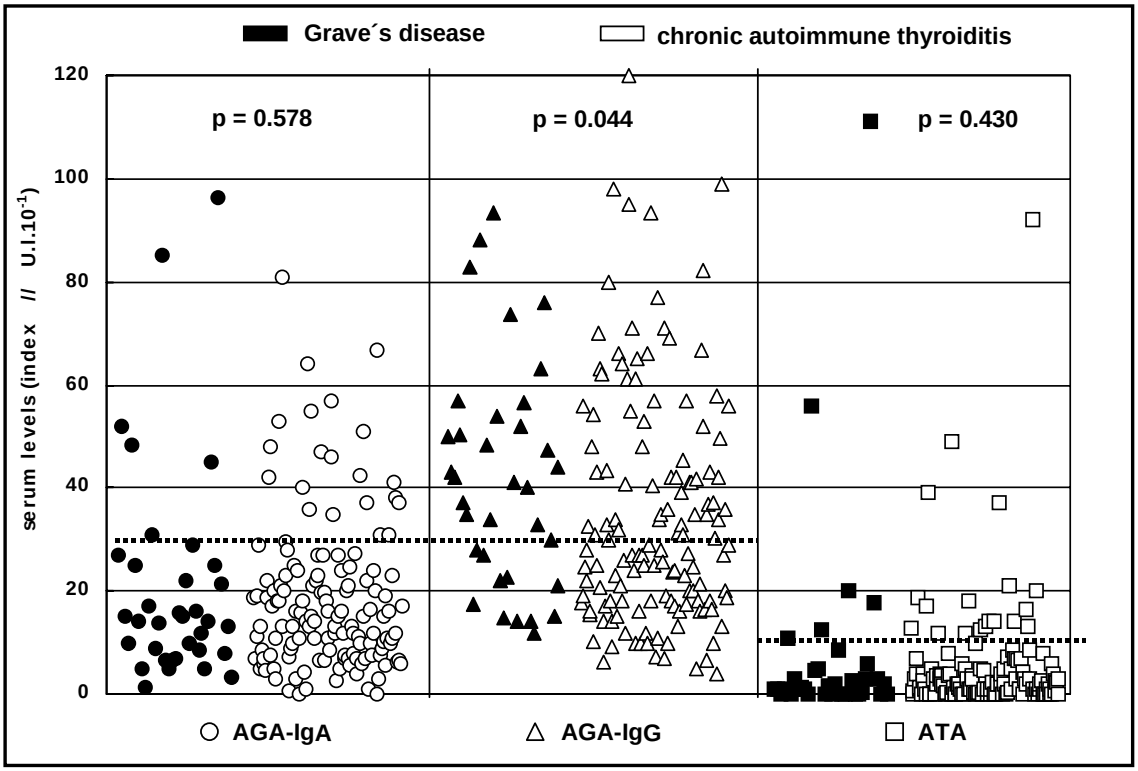

Fig. 2. *Antigliadin and anti-tissue transglutaminase antibodies in patients with autoimmune thyroid diseases. AGA-IgA, AGA-IgG: serum levels of IgA and IgG antigliadin antibodies (results were related to the laboratory standard and expressed as an index), ATA: serum levels of IgA anti-tissue transglutaminase antibodies (U.l.10^{-1}), p: level of significance (Mann-Whitney test).*

68.57 % GD). Positivity of AGA-IgA, AGA-IgG and ATA was found in 3.55 % with AITD (2.99 % CT and 5.71 % GD), compared to 0.68 % in the control subjects ($p<0.05$), positivity of AGA-IgA and AGA-IgG in 11.83 % patients with AITD (10.45 % CT and 17.14 % GD), compared to 2.20 % in the control group ($p<0.001$). Positivity of AGA-IgA and ATA was ascertained in 4.14 % patients with AITD (3.73 % CT and 5.71 % GD), compared to 0.83 % in the control subjects ($p=0.001$), positivity of AGA-IgG and ATA in 9.77 % with AITD (8.21 % CT and 14.29 % GD), compared to 0.68 % in control subjects ($p<0.001$). The prevalence of positivity and the medians of serum concentrations of AGA-IgA, AGA-IgG, ATA and AEA in patients with AITD did not significantly differ in age categories, and we did not discover a significant difference in serum titres of antibodies in untreated persons and those who became euthyroid.

Patients with chronic thyroiditis treated with a high replacement dosage of levothyroxin (125-200 μg daily) had significantly higher serum levels of AGA-IgA in comparison with persons treated with a lower dosage (50-100 μg daily) (medians: 19.69 vs. 13.00, $p=0.033$). Likewise, the medians of serum levels and the percentage of positivity of AGA-IgG and ATA were higher in the group with a higher daily replacement dosage; these differences were not statistically significant (Fig. 3).

We found a significantly negative correlation of serum concentrations of ATA and total serum calcium ($r = –0.480$, $p=0.0236$, $n=22$) (Fig. 4) and a borderline significant positive correlation of ATA and CRP ($r = 0.654$, $p=0.05$, $n=9$). A significant negative correlation was discovered between serum concentrations of AGA-IgA a TSH in persons with GD ($r = –0.426$, $p=0.0169$, $n=35$).

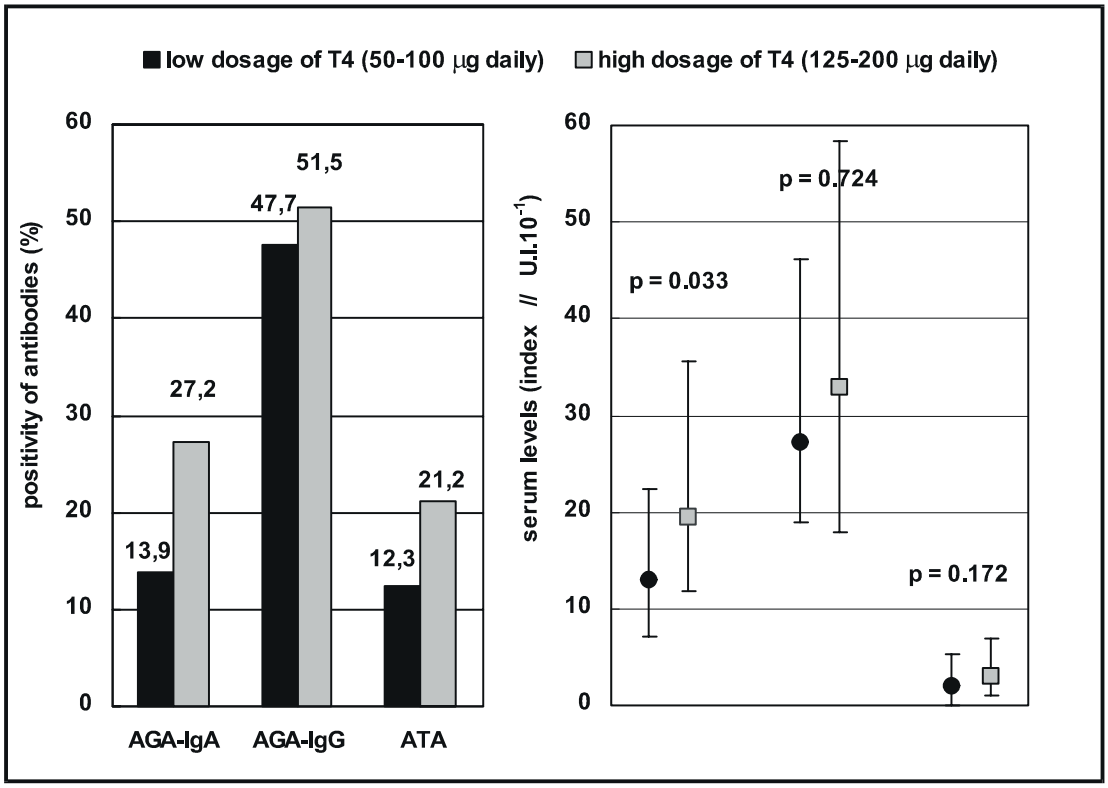

Fig. 3. *Relationship between positivity of antigliadin and anti-tissue transglutaminase antibodies and levothyroxin replacement dosages in patients with treated chronic autoimmune thyroiditis. AGA-IgA, AGA-IgG: serum levels of IgA, IgG antigliadin antibodies (results were related to the laboratory standard and expressed as an index), ATA: serum levels of IgA anti-tissue transglutaminase antibodies (U.l.10^{-1}), p: level of significance (Mann-Whitney test), T4: therapeutically administered levothyroxine.*

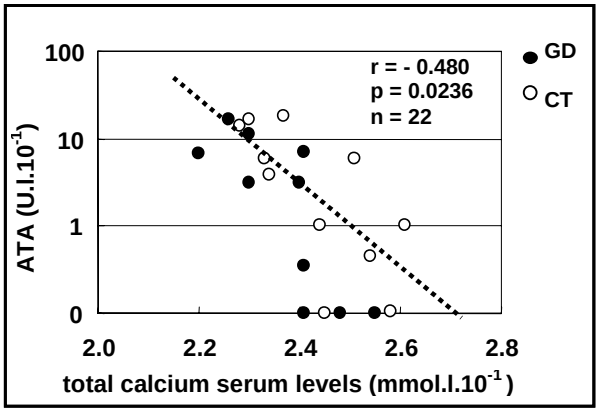

Fig. 4. *Negative correlation between anti-tissue transglutaminase antibodies and total calcium serum levels. ATA: serum levels of IgA anti-tissue transglutaminase antibodies (U.l.10^{-1}), GD: Graves´ disease (hyperthyroid or euthyroid during carbimazol therapy or after total thyroidectomy), CT: chronic autoimmune thyroiditis (euthyroid or hypothyroid), r: Spearman rank order correlation coefficient, p: level of significance, n: number.*

We did not find any significant relation between TPO-Ab and TGB-Ab, and AGA-IgA, AGA-IgG and ATA. There was no relationship between the level of titres of AGA-IgA, AGA-IgG and ATA and the sonographically documented volume of the thyroid gland. In subjects with CT, the volume of the thyroid gland in milliliters positively correlated with the level of the titre of TPO-Ab (r=0.372, p=0.0185, n=40).

Discussion

Gliadins, which represent storage proteins of wheat grain, are considered one of the main antigens which are of importance in the pathogenesis of celiac disease. Similar proteins appear likewise as storage materials of rye, barley, and oats and are grouped together as prolamins. They all share repeating sequences with high proline and glutamine content. Their mobility in starch electrophoresis was our means of distinguishing several groups of gliadins (α-, β-, γ-, δ-gliadins). Gliadins

act as antigens and cause an immunopathological Th1 and Th2 responses, accompanied by the production of Th1 and Th2 cytokines with regulatory and effector roles. According to experimental work (Maiuri *et al.* 2000, 2001), interleukin-15 plays an important role in the pathogenesis of celiac disease. In certain circumstances (for instance genetic disposition, disorders in intestinal immunity, disorders of intestinal permeability), gliadin penetrates into the subepithelium. After its first contact with immunocompetent cells (antigen presenting cells and intraepithelial T lymphocytes) in the intestine, the mucosal lymphoid tissue is activated and the progression of immunopathological process occurs. The result of this is an increase in intestinal permeability (Johnston *et al.* 2001, Perry *et al.* 2001) and a closing of the vicious circle. Activated immunocompetent cells move off from the area of the first contact into intestinal circulation and enter further areas of the gastrointestinal tract after the mutual linkage of several adhesive molecules. The $\alpha 4\beta 7$ lymphocyte integrin enters into contact with MAdCAM-1 endothel vessels and $\alpha E\beta 7$ lymphocyte integrin makes contact with the E-cadherin in the intestinal epithelial cells (this process is called "homing") (Dando *et al.* 2002). Interesting experimental work revealed the expression of $\alpha 4$ integrins in activated lymphocytes, which originate in the thyroid gland affected by autoimmune thyroiditis (McMurray *et al.* 1996). One attractive hypothesis assumes the abnormal targeting of immunocompetent cells even outside the gastrointestinal tract (GIT) and the induction of autoimmune reaction in the other organs, above all the endocrine glands. This hypothesis is also supported by the ontogenetic relatedness of the gastrointestinal and endocrine systems. According to several studies, the catalytic antigen might indeed be gliadin. Tissue transglutaminase was identified as the main autoantigen of celiac disease and the antigen of antiendomysial antibodies (Dieterich *et al.* 1997, 1998). Tissue transglutaminase is a calcium-dependent enzyme which catalyses the transamide reaction in glutamine remnants of the polypeptide gliadin chain with the emergence of new antigen epitopes which induce the autoimmune reaction in genetically predisposed individuals.

Overt celiac disease occurs most often in children; in adults the subclinical and oligosymptomatic forms are more frequent (Murray 1999). The clinical symptomatology is astrointestinal (abdominal distention, abdominal pain, diarrhea, steatorrhea, lactase deficiency) and systemic – caused by resorption disorders for various substances (weight loss, fatigue, iron, folate and vitamin B_{12} deficiency, osteoporosis, osteomalatia, vitamin K deficiency, infertility, polyneuropathy, aphthous stomatitis, arthralgia, epilepsy, depression, alopecia). Particular manifestation of celiac disease is dermatitis herpetiformis Duhring (Keaveny *et al.* 1996, Murray 1999, Farrell *et al.* 2002). A negative correlation of ATA and total calcium serum levels, ascertained in part of our group (22 subjects with determined calcium levels) may be a symptom of a subclinical resorption disorder for calcium and vitamin D. The most frequent manifestation of celiac disease in adulthood occurs at the age between 30 and 40 years, often during difficult junctures (pregnancy, trauma, infection, stress, surgery). In our group we did not find any significant relationship between antibodies associated with celiac disease and age. The prevalence of clinically overt and bioptically verified forms of celiac disease is given at approximately 1:1000 (the highest is found in Northern Europe and Ireland – 1:300) (Catassi *et al.* 1995). The actual occurrence of subclinical and oligosymptomatic forms of the illness is, however, probably much higher and is estimated to be at about 1:200-1:250 (Not *et al.* 1998). Up to 80 % of illnesses thus remain undiagnosed, and this can have serious medical consequences for the people affected. On the basis of these observations, the iceberg model of celiac disease was defined: 1) overt disease (mucosal histopathological changes, resorption abnormalities, clinical symptoms, antibodies positivity), 2) subclinical disease (mucosal histopathological changes, resorption abnormalities, antibodies positivity, without clinical symptoms), 3) silent disease (genetic predisposition, antibodies positivity, possible resorption abnormalities, without histopathological mucosal changes and clinical features), and 4) potential disease (only genetic predisposition) (Ferguson *et al.* 1993, Catassi *et al.* 1996).

Some clinical studies have demonstrated a significant association between autoimmune thyroid diseases and celiac disease. If there exists a functional pathogenetic relationship between both illnesses or whether it is only a case of disposition to crossed autoimmune reactivity in persons with AITD, manifested in the positivity of various antibodies without any further clinical correlate, is not as yet known. According to studies so far, 14-20.5 % of people with celiac disease also have AITD (Counsell *et al.* 1994, Sategna-Guidetti *et al.* 1998), 21 % have positive microsomal thyroid antibodies diagnosed with the immunofluorescent method (Volta *et al.* 1997), and 30 % of patients have positive TPO-Ab and sonographic patterns of AITD (Velluzi *et al.*

1998). In 12.9 % of subjects with celiac disease, hypothyroidism caused by AITD was reported, and in 16.2 % of people AITD with normally functioning thyroid glands was diagnosed (Sategna-Guidetti *et al.* 2001).

On the other hand, bioptically verified celiac disease occurs in 3.2-4.8 % of people with AITD (Volta *et al.* 2001, Valentino *et al.* 1999a, Sategna-Guidetti *et al.* 1998, Cuoco *et al.* 1999, Collin *et al.* 1994), compared to 0.4 % in healthy individuals (Volta *et al.* 2001, Collin *et al.* 1994). The prevalence of AEA positivity in patients with AITD is about 3.3 % (Sategna-Guidetti *et al.* 1998, Valentino *et al.* 1999a,b), Volta *et al.* 2001), which is somewhat higher than we discovered in our group (1.18 %). An isolated study gives the prevalence of positivity of ATA at about 2.7 % subjects with AITD (Volta *et al.* 2001), but according to our results it is notably higher (14.79 %). There is inconsistent information about AGA-IgA and AGA-IgG in patients with AITD; according to our results, there is a prevalence of positivity in patients with AITD 15.98 % for AGA-IgA and 51.48 % for AGA-IgG.

In agreement with published reports we found that the prevalence of all types of antibodies is high in patients with AITD. Two persons (1.18 %) had all four antibodies positive, and had mild GIT difficulties (abdominal distention, abdominal pain), that disappeared after the implementation of a gluten-free diet. Clinical celiac disease was confirmed in these subjects by enterobiopsy. In 3.55 % of patients (2.99 % with CT and 5.71 % with GD) three antibodies were positive (AGA-IgA, AGA-IgG and ATA), and negative AEA, and in 4.14 % (3.73 % CT and 5.71 % GD) AGA-IgA and ATA were positive. Although these persons had no GIT symptoms, the subclinical form of celiac disease with resorption disorders is likely to be found here, and they should be examined for several markers of intestinal resorption (e.g, beta-caroten serum levels, intestinal permeability test) and optionally advised to enterobiopsy. In 14-17 % of people with one type of antibody positive (AGA-IgA or ATA) latent celiac disease, or a genetic predisposition, may also be present; alternatively, antibody positivity in these subjects is merely an expression of raised crossed autoimmune reactivity. In comparison with the control sera, the positive AGA-IgG were found to be surprisingly high in persons with AITD with a significantly higher prevalence in the GD group (65.7 %) as opposed to the CT group (47.8 %). The significance of this finding remains unclear. It is possible that it is only a case of anamnestic antibodies whose positivity lasts beyond contact with gliadin in healthy people, or whose positivity is connected with raised crossed autoimmune reactivity. AGA-IgG may have a diagnostic significance in people with an IgA deficiency.

It is not yet known if there is a difference in the occurrence of celiac disease in individual AITD types. We revealed a higher prevalence of antibodies positivity in people with GD compared to CT and significantly higher serum AGA-IgG levels in patients with GD compared to CT. In agreement with this, in patients with GD there was a significant negative correlation of AGA-IgA and TSH, although those results are of borderline significance because of the different sizes of the groups examined.

Several studies indicated that impaired thyroid function in patients with untreated celiac disease improves after the implementation of a gluten-free diet, and a drop in the titre of thyroid antibodies was observed. A normalization of the thyroid function or a lowering of required replacement dosages of levothyroxin was observed in patients with celiac disease and hypothyroidism after implementation of gluten-free diet (Sategna-Guidetti *et al.* 2001, Valentino *et al.* 1999a,b). Yet another study describes a fall in titres of antiendomysial antibodies, thyroid antibodies and antibodies associated with type 1 diabetes mellitus in patients with celiac disease after the implementation of a gluten-free diet (Ventura *et al.* 2000). Certain authors believe that a gluten-free diet in people with celiac disease (along with a genetic disposition to the development of autoimmune insulitis) can delay or even fully prevent the manifestation of type I diabetes mellitus (Kumar *et al.* 2001). Likewise, a significantly higher prevalence of positive thyroid and glutamic acid decarboxylase antibodies and lower thyroxin serum levels were observed in children with untreated celiac disease, compared to those treated with gluten-free diet (Karczewska *et al.* 1992, Toscano *et al.* 2000).

Our results confirm that patients with higher thyroid replacement requirements also have higher antibody titres, especially AGA-IgA. On the basis of this fact, we can draw two conclusions: 1) impaired intestinal immunity can lead to the initiation or progression of thyroidal autoimmune disorders with low production of hormones, and 2) an impaired resorption of various substances may occur (for levothyroxine in persons undergoing replacement therapy, for iodine, selenium, calcium, vitamin D, iron etc).

The verification of these findings will require to expand the group studied and to monitor especially the

effects of gluten-free diet implementation in patients with dyspeptic difficulties, symptoms of malabsorption and higher antibody titres.

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