

# Immunologic and Biochemical Factors of Coincident Celiac Disease and Type 1 Diabetes Mellitus in Children

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**ABSTRACT:** The objective of the study was to investigate whether immunologic and biochemical events occurring in the course of type 1 diabetes mellitus might play a role in the development of the celiac disease. The study was carried out on 223 children with long-standing diabetes mellitus type 1 (DM1). All the patients had TSH, fT4, fT3, urinary albumin secretion rate, IgA, level of antigliadin antibodies (AGA) IgA and IgG, antitissue transglutaminase IgA antibodies, antiendomysium (EmA) IgA and IgG antibodies and antityreoglobulin antibodies, antithyroid peroxidase antibodies evaluated. Serum TNF- $\alpha$ , IL-6, and IL-10 levels were also measured. The group of children with coincident DM1 and celiac disease and without autoimmune thyroiditis was characterized by significantly higher glycosylated hemoglobin, higher serum TNF- $\alpha$ , IL-6 but lower serum IL-10 in relation to the remaining diabetic patients. A statistically significant positive correlation was observed between IgA-anti-tTG and serum TNF- $\alpha$  ( $R = 0.28, p = 0.026$ ); between IgG AGA and serum IL-6 ( $R = 0.31, p = 0.023$ ); and between glycosylated hemoglobin and IgA-anti-tTG ( $R = 0.21, p = 0.001$ ) and IgA antiendomysium ( $R = 0.22, p = 0.001$ ). Poor metabolic control, persistent elevated levels of proinflammatory cytokines, and decreased level of antiinflammatory cytokines occurring in the course of type 1 diabetes mellitus might influence the incidence of celiac disease. (*Pediatr Res* 64: 677–681, 2008)

Various publications report correlation between diabetes mellitus type 1 (DM1) and incidence of other autoimmune disorders, particularly thyroid and celiac diseases (1). According to various data, 1.0–10.4% of children diagnosed with DM1 develop celiac disease—the data vary according to age and diagnostic methods (2). In Northern Poland, celiac disease was present in 5.7% of children at the onset of DM1. Conversely, 9.4% of children with long-standing DM1 had concomitant celiac disease (3). Although numerous works regarding the coincidence of DM1 and celiac disease were published in recent years, the ethiopathogenesis of celiac disease development in diabetic patients is not fully understood. One argument in favor of common inclination is the fact that the same alleles of the genes HLA-DQA1 and DQB1 predispose for development of both celiac disease and DM1 (4,5). However, the association of the two autoimmune diseases with the same HLA region only partially provides

evidence for the existence of genetic predisposition and the similarities in the pathogenesis of the auto-aggression reactions, especially in the patients, who do not have the HLA-DQA1\*0501 and DQB1\*0201 heterodimer.

The question arises, whether the disturbances of the equilibrium between subpopulations of helper lymphocytes: T<sub>H</sub>1 and T<sub>H</sub>2 as observed in children with DM1 might lead toward development of celiac disease in this particular group of patients (6). Our previous studies have shown that TNF- $\alpha$  and IL-6 play crucial role in the mechanisms of damage to the eye apparatus and kidneys of the diabetic patients (7–10). It remains unresolved to what extent changes in the levels of pro- and antiinflammatory cytokines that occur in the course of DM1 might affect occurrence of celiac disease in such patients.

This work aimed to assess the correlation between the levels of TNF- $\alpha$ , IL-6, IL-10 and celiac disease development in children diagnosed with DM1.

## MATERIALS AND METHODS

The study was conducted on a group of 223 children with long-standing DM1 (105 boys, 118 girls) aged  $12.20 \pm 3.87$  y; the duration of the disease being  $4.47 \pm 3.16$  y. All of the children were patients of the Outpatient Diabetology Clinics and the Department of Pediatrics, Hematology, Oncology and Endocrinology at the Medical University of Gdańsk, Poland. The diagnosis of DM1 was made in accordance with the American Diabetes Association Criteria (11).

All the patients were treated using humanized insulin. In children with long-standing DM1, the average daily insulin dose was  $0.93 \pm 0.35$  unit/kg. The disease metabolic compliance was assessed based on the level of glycosylated Hb (HbA1c) measured in the blood by immunoturbidimetric method using a Unimate 3 set (Hoffmann-La Roche AG, Germany). C-reactive protein level was measured using a highly sensitive test (HsCRP).

Every patient, while being hospitalized in the Department in the years 2003–2005, was subject to all of the celiac disease screening tests. These included detection of antigliadin antibodies in both IgA and IgG classes (IgA-AGA/IgG-AGA), antitissue transglutaminase IgA antibodies (IgA-anti-tTG), and antiendomysium IgA and IgG antibodies (IgA-EmA/IgG-EmA). Additionally, the total level of IgA was measured to exclude cases of IgA deficiency. Final diagnosis of celiac disease depended on the results of jejunal biopsy, in accordance with criteria set by European Society for Pediatric Gastroenterology and Nutrition (12,13).

Biopsy samples were obtained through an endoscopy procedure using an Olympus gastroscope (Tokyo, Japan) with four to five specimens being taken from the second portion of the duodenum. All biopsy specimens were blindly

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**Abbreviations:** AGA, antigliadin antibodies; CV, mean variability coefficient; DM1, diabetes mellitus type 1; EmA, antiendomysium antibodies; HbA1c, glycosylated hemoglobin; TG, tyreoglobulin; TPO, thyroid peroxidase; tTG, tissue transglutaminase

evaluated by a pathologist and a gastroenterologist and classified according to Marsh classification.

To detect thyroid disorders, all the patients were evaluated with regard to hormone studies (TSH, fT4, fT3), levels of antitireoglobulin (anti-TG) and antithyroid peroxidase (anti-TPO) antibodies, and results of thyroid ultrasound examination.

The EmA antibodies were measured by means of indirect immunofluorescent method that was performed on cryostat fragments of the human umbilical cord tissue. The first layer consisted of serum from the examined case, whereas the second contained rabbit immunoserum, labeled with FITC, directed against human EmA class IgA and class IgG (purchased from DAKO, Glostrup, DK). The cases, in which for the first layer PBS or a known EmA positive serum was used, were used as controls. The results were analyzed by fluorescent microscopy. Titers lower than 1:10 were judged to be the negative threshold. IgA-anti-tTG, IgA-AGA/IgG-AGA, anti-TG, anti-TPO antibodies were detected by means of an immunoenzymatic method (ELISA). ELISA tests were performed using commercial kits purchased from Hycor Biomedical, Inc., San Diego, CA, and Orgentec Diagnostics, Germany. The final result of the patient was calculated as mean value of two measurements. The reference values for examined antibodies were IgA-anti-tTG <7 U/mL [mean variability coefficient (CV) for analyzed cases and controls -3.08%], IgA-AGA <12 U/mL (CV -5.0%), IgG-AGA <12 U/mL (CV -4.4%), anti-TG <100 IU/mL (CV -1.45%), anti-TPO <50 IU/mL (CV -2.57%).

Additionally, all the examined children had TNF- $\alpha$ , IL-6, and IL-10 serum levels measured using immunoenzymatic ELISA method (Quantikine High Sensitivity Human by R&D Systems, Minneapolis, MN) according to the manufacturer's protocol. Minimum detectable concentrations were determined by the manufacturer as 0.12 pg/mL, 0.03 pg/mL, and 1.0 pg/mL, respectively. Intraassay (2.6 for TNF- $\alpha$ ; 1.6 for IL-6; 1.7 for IL-10) and interassay (7.4 for TNF- $\alpha$ ; 6.4 for IL-6; 7.5 for IL-10) precision's performances of the assays were determined on 20 replicates from the quality control data of the laboratory.

**Blood collection.** Blood samples were collected from 7 to 9 o'clock after an overnight fast. The sera were separated from the venous blood within 30 min and kept frozen at -80°C up to 3 mo before analysis. All measurements were done on the same blood sample.

Patients with long-standing DM1 were subdivided into two groups: children with DM1 and celiac disease but without autoimmune thyroiditis and without microalbuminuria (group A;  $n = 19$ ) and children with DM1 but without celiac disease, autoimmune thyroiditis, and microalbuminuria (group B;  $n = 160$ ). Group B was regarded as a control group. The DM1 patients, who on the basis of clinical, biochemical, laboratory, hormonal, and immunologic studies were diagnosed with urinary, liver, respiratory disease, or any disorder of autoimmune origin other than DM1 were excluded from group A and B.

Parents of all of the children in the study gave informed consent. In addition, positive recommendation from Independent Bioethical Committee for Scientific Research at Medical University of Gdańsk (NKEBN/610/2003/2004) was obtained.

**Statistical analysis.** The results were analyzed using Statistica 7.0 program (StatSoft, PL). The Kolmogorov-Smirnov test was used to evaluate normality of variables. Logarithmic transformation was applied to variables as required. The differences between the groups were calculated with  $t$  test or the nonparametric Mann-Whitney U tests. The Spearman or the Pearson correlations were examined. In this study, a value of  $p < 0.05$  was considered statistically significant.

## RESULTS

Twenty-one (9.4%) of 223 examined DM1 patients were diagnosed to have celiac disease based on positive result of jejunal biopsy. Additionally, 44 (19.7%) patients were diagnosed with autoimmune thyroiditis.

Children with long-standing DM1 and diagnosed celiac disease (group A) were significantly older at diagnosis of DM1 ( $p = 0.003$ ), and were characterized by significantly higher level of HbA1c ( $<0.001$ ), CRP ( $<0.001$ ), and 24 h urinary albumin secretion ( $p = 0.013$ ). No statistically significant differences between the two groups in thyroid hormone levels and BMI were observed (Table 1).

Significantly higher mean level of IgA-AGA ( $p < 0.001$ ), IgG-AGA ( $p < 0.001$ ), IgA-EmA ( $p < 0.001$ ), IgG-EmA

**Table 1.** Clinical and biochemical parameters of children with diabetes mellitus type 1 and celiac disease (group A) and children with diabetes mellitus type 1 and no concurrent celiac disease (group B)

Parameters	Group A ( $n = 19$ )	Group B ( $n = 160$ )	$p$
Age (y)	11.15 $\pm$ 4.34	11.06 $\pm$ 4.25	0.870
Sex (F/M)	15/4	83/77	0.053
Duration of diabetes (y)	3.24 $\pm$ 3.11	6.10 $\pm$ 3.22	0.021*
BMI (kg/m <sup>2</sup> )	18.63 $\pm$ 3.30	18.00 $\pm$ 3.11	0.338
Dose insulin (unit/kg/24 h)	0.89 $\pm$ 0.31	0.92 $\pm$ 0.28	0.451
C-peptide-serum (ng/mL)	<0.05	<0.05	—
HbA1c-serum (%)	9.49 $\pm$ 2.13	8.26 $\pm$ 1.97	0.001*
Albumin excretion rate (mg/24 h)	12.8 $\pm$ 11.6	6.20 $\pm$ 5.80	0.013*
CRP-serum (mg/L)	3.36 $\pm$ 1.47	1.08 $\pm$ 0.82	0.001*
TSH ( $\mu$ U/mL)	2.02 $\pm$ 0.62	1.93 $\pm$ 0.99	0.843
FT4 (pmol/L)	15.02 $\pm$ 2.41	14.57 $\pm$ 2.50	0.805
FT3 (pmol/L)	3.83 $\pm$ 0.92	5.53 $\pm$ 0.22	0.818

\* Statistical significance ( $p < 0.05$ ).

**Table 2.** Immunologic parameters of children with diabetes mellitus type 1 and celiac disease (group A) and children with diabetes mellitus type 1 and no concurrent celiac disease and autoimmune thyroiditis (group B)

Immunological parameters	Group A ( $n = 19$ )	Group B ( $n = 160$ )	$p$
IgA-anti-tTG (U/mL)	85.37 (0.63–239.95)	3.40 (0.00–28.02)	<0.001*
IgA AGA (U/mL)	51.01 (0.63–258.70)	3.30 (0.00–40.00)	<0.001*
IgG AGA (U/mL)	34.94 (1.10–170.50)	5.73 (0.005–42.50)	<0.001*
IgA EmA-positive patients ( $n$ )	11/19	0/160	<0.001*
IgG EmA-positive patients ( $n$ )	3/19	0/160	<0.001*
IgA (g/L)	2.16 (2.08–2.63)	2.12 (1.98–2.58)	0.323
Serum TNF- $\alpha$ (pg/mL)	2.65 (1.10–4.75)	0.41 (0.00–1.80)	<0.001*
Serum IL-6 (pg/mL)	2.36 (0.35–4.36)	0.50 (0.00–2.13)	<0.001*
Serum IL-10 (pg/mL)	0.73 (0.00–3.47)	1.69 (0.00–4.70)	0.03*

\* Statistical significance ( $p < 0.05$ ).

( $p < 0.001$ ), IgA-anti-tTG ( $p < 0.001$ ) antibodies as well as TNF- $\alpha$  and IL-6 serum values ( $p < 0.001$ ;  $p < 0.001$ ) were found in group A in relation to group B. However, serum IL-10 in the group A was significantly lower than in the group B (Table 2).

The group of children with DM1 and celiac disease ( $n = 19$ , duration of diabetes: 3.24  $\pm$  3.11 y) when compared with the group of diabetic patients without celiac disease but of similar duration of the disease ( $n = 129$ , duration of diabetes: 3.41  $\pm$  2.98 y) had statistically significant differences in the cytokine levels. TNF- $\alpha$  ( $p < 0.001$ ) and IL-6 ( $p < 0.001$ ) were significantly higher whereas IL-10 ( $p = 0.02$ ) was significantly lower in the group of diabetic patients diagnosed to have concomitant celiac disease.

In all the examined children with DM1, the C-peptide level was found to be less than 0.5 ng/mL.

**Table 3.** Correlation between biochemical and immunological parameters in children with diabetes mellitus type 1

Biochemical and immunological parameters	R	p
HbA1c vs IgA-anti-EmA	0.22	0.001
HbA1c vs IgA-anti-tTG	0.21	0.001
CRP vs IgA-anti-EmA	0.26	0.000
CRP vs IgG-anti-EmA	0.22	0.014
CRP vs IgA-anti-tTG	0.16	0.016
TNF vs IgA-anti-tTG	0.28	0.026
IL-6 vs IgA AGA	0.31	0.023
IL-10 vs IgA-anti-tTG	-0.27	0.015

**Table 4.** Sensitivity and specificity of serologic test for celiac disease detection in DM1 children

Serologic test	Sensitivity (%)	Specificity (%)
IgA anti-tTG	95.2	95.0
IgA AGA	76.2	84.1
IgG AGA	80.4	88.3
IgA EmA	61.9	100.0
IgG EmA	68.5	78.2

**Correlation between biochemical and immunologic parameters in children with DM1.** There was a statistically significant positive correlation between CRP levels and IgA-anti-tTG ( $R = 0.16$ ,  $p = 0.016$ ) and both classes of EmA antibodies: IgA ( $R = 0.26$ ,  $p = 0.000$ ) and IgG ( $R = 0.17$ ,  $p = 0.014$ ) observed in children with DM1. Additionally, significant positive correlation between HbA1c and IgA-anti-tTG ( $R = 0.21$ ,  $p = 0.001$ ) and IgA EmA ( $R = 0.22$ ,  $p = 0.001$ ) was found. There was also a statistically significant positive correlation between IgA-anti-tTG and serum TNF- $\alpha$  ( $R = 0.28$ ,  $p = 0.026$ ) and IgG AGA and serum IL-6 ( $R = 0.31$ ,  $p = 0.023$ ). Finally, significant negative correlation between IgA-anti-tTG and serum IL-10 ( $R = -0.27$ ,  $p = 0.015$ ) was observed (Table 3).

**Sensitivity assessment of the tests for celiac disease diagnosis in DM1 children.** The highest sensitivity (95.2%) of celiac disease detection in DM1 children was found in the case of serologic test detecting anti-tTG (IgA tTG), whereas the lowest sensitivity characterized test aimed at antiednomy-sium antibody (IgA EmA) detection (61.9%). However, the specificity of both tests was comparable—IgA tTG-95%, IgA EmA-100% (Table 4).

## DISCUSSION

The pathomechanism of the celiac disease development in DM1 children remains, for the most part, unknown. Published studies do not give a clear answer whether immunologic disorders observed in DM1 children with concomitant celiac disease are of primary character and thus directly related to the ethiopathogenesis of DM1, or possibly they result simply secondarily to direct exposition to gluten.

A few published reports indicate that gluten might influence autoimmunologic response against pancreatic  $\beta$  cells. For instance, Funda *et al.* (14) have observed that after treatment with a gluten-free diet, BB-line rats eliminated antibodies characteristic for diabetes. Conversely, Hummel *et al.* (15) did

not observe either alterations of the levels of auto-antibodies or decrease in the risk of diabetes development after introduction of a gluten-free diet in the relatives of the DM1 patients. Inconsistent results of the aforementioned studies suggest that the relationship between DM1 and celiac disease is of complex character.

Gluten found in everyday diet appears to be a modulator and not a determining factor in DM1 ethiopathogenesis. It allows other factors, which in patients with genetic predisposition to DM1 development might activate autoimmune response against pancreatic  $\beta$  cells to have better access to *lamina propria* of the intestinal mucosa (16).

Authors of a few published studies suggest that immunologic processes responsible for destruction of pancreatic  $\beta$  cells at an early stage of DM1 development persist during the chronic stage of the disease and might influence the development of late complications, including diabetic nephropathy and retinopathy (17–19). Our results have confirmed increased activity of TNF- $\alpha$  and IL-6 and decreased activity of IL-10 in DM1 patients, especially among children who had the course of disease complicated by occurrence of pathologic events in the kidneys and eye apparatus (7).

Observed elevated serum levels of TNF- $\alpha$  and IL-6 and decreased level of IL-10 in DM1 children with coexistent celiac disease when compared with the patients without confirmed celiac disease suggest that these cytokines play a significant role in celiac disease development among DM1 children. Elevated levels of proinflammatory cytokines observed in the course of DM1 might account not only for damage to kidneys and eye apparatus but also for impairment of intestinal mucosa.

Statistically significant correlation between TNF- $\alpha$  and IgA-anti-tTG, as shown in our work, might suggest that TNF- $\alpha$  is among major factors responsible for damage to the small intestine epithelium and increase in tTG secretion (20). Recently, a few studies were published on the role of antibodies against tTG (anti-tTG) in celiac disease diagnosis (21). Our studies provide further evidence that IgA-anti-tTG antibodies, among the other so far analyzed antibodies, may become the most sensitive screening indicator of latent celiac disease in DM1 children.

The enzyme, by means of deaminase mechanism, modifies the structure of gliadin, which consequently becomes more immunogenic (21). Moreover, TNF- $\alpha$  working together with IL-6 increases expression of HLA-DQ genes in the intestinal epithelium allowing for more gliadin to be presented to the intestinal mucosa's T lymphocytes (22).

Some authors claim that early detection of celiac disease in DM1 children is of considerable clinical significance. The introduction of gluten-free diet in patients with coexistent DM1 and celiac disease might allow patients in future to reach adequate height, to achieve puberty, and to have better metabolic compliance of DM1 (23). It might also prevent diabetic microangiopathy development (24). However, some published reports show no observable effect of better metabolic compliance of DM1 while on a gluten-free diet (25). In such studies, the occurrence of celiac disease in DM1 children appears to be only partially triggered by direct exposure to gluten. This begs

the question: how much does poor metabolic control of DM1 influence the prevalence of celiac disease in those patients? The results of our studies show significantly higher HbA1c levels and significantly increased 24 h urinary albumin secretion in children with DM1 and concomitant celiac disease in relation to diabetic patients without coexistent celiac disease.

Our study has shown statistically significant positive correlation between serum TGA and HbA1c levels. These results suggest that poorer metabolic compliance of DM1 is related to higher risk of celiac disease development in DM1 children. In the course of DM1, hyperglycemia plays a crucial role in initiation and progression of tissue damage by nonenzymatic glycation (26). Glycated proteins when binding to their specific receptors located on the cell membrane of the macrophages trigger release of TNF- $\alpha$ , IL-6, IL-12, and IL-2 cytokines (26,27). These multifunctional cytokines may modulate inflammatory response in the intestinal mucosa and thus take part in its impairment and celiac disease development in DM1 children (28).

Increasing number of evidence suggest that the starting point for the development of the inflammatory reaction in the intestinal mucosa is not only the increase in TNF- $\alpha$  and IL-6 production but also inadequate low production of the anti-inflammatory cytokines aimed at reduction of the neurotoxic effect of the proinflammatory cytokines (27,29). Animal models show that depletion of regulatory TCD4<sup>+</sup>CD25<sup>+</sup> lymphocytes producing IL-10 in mice causes spontaneous development of enterocolitis (30). However, improvement in the medical condition of chronic enterocolitis was observed after administration of the exogenous IL-10 (31,32). Such a therapy blocked excessive secretion of TNF- $\alpha$  and INF $\gamma$  (33). In the available literature, no work that focused on the assessment of IL-10 levels in children with DM1 and concomitant celiac disease was found. Our previously published studies have shown that there is a shortage of IL-10 in children with DM1, especially among children who had the course of disease complicated by the occurrence of pathologic events in the kidneys and the eye apparatus (7). In the current study of 19 children with DM1 and diagnosed with concomitant celiac disease, as many as 14 (74%) children did not present any activity of this cytokine in the blood serum. Preliminary analysis of the IL-10 level in the group of examined children with DM1 and celiac disease suggests that higher secretory activity of IL-10 might have a protective effect thus preventing celiac disease development. Introducing IL-10 to the anti-inflammatory therapeutic protocols of other disorders of the digestive tract, including colitis ulcerosa, was a therapeutic success (34,35). Further research to assess the negative influence of IL-10 depletion on celiac disease development in children with DM1 may allow for future introduction of similar therapies to increase the IL-10 level in this particular group of patients.

In addition, the results of our research on the role played by TNF- $\alpha$  encourage efforts aimed at early introduction of immunologic treatment to inhibit TNF- $\alpha$  activity. In recent years, several studies were published on the role of TNF- $\alpha$  in promoting inflammatory processes in the course of chronic intestinal diseases (36). Numerous pharmacologic protocols to

inhibit TNF- $\alpha$  activity (including infliximab, CDP571, and MAb) are currently carried out worldwide (37–40).

From our data, TNF- $\alpha$  appears to be an essential factor of celiac disease development. Possibly, early introduction of TNF- $\alpha$  antagonists to the treatment of DM1 patients in whom elevated levels of this cytokine were detected might prevent celiac disease development and late diabetic complications. In addition, in patients already diagnosed with celiac disease, especially if few effects of the gluten-free diet are observed, it might help to obtain metabolic compliance of the diabetes.

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