

Seroprevalence of celiac disease in Belgian children and adolescents

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Abstract

Background : Celiac Disease (CD) has a high prevalence worldwide. Because the disease may be underdiagnosed in Belgium, we wanted to evaluate the seroprevalence of CD in children and adolescents.

Patients and methods : Serum samples from 1159 apparently healthy children and adolescents aged between 1-19 years, were prospectively collected in 2006 by 15 Belgian diagnostic laboratories. In September 2009, all samples were analyzed for human tissue transglutaminase IgA antibodies (IgA tTG) and total IgA levels by, respectively a commercial ELISA and immunonephelometry. Sera, found positive, were assessed by immunofluorescence for the presence of IgA and IgG antiendomysium antibodies (IgA/IgG EMA). In patients with IgA deficiency IgG antibodies against deamidated gliadin peptides (IgG DGP) and IgG antiendomysium antibodies (IgG EMA) were determined.

Results : Ten of the 1159 individuals (0.86%) tested positive for IgA tTG. A further 0.86% showed borderline IgA tTG results. In almost two percent (1.98%) of the analyzed samples total IgA levels below the lower limit of normal were observed. Four out of eight positive IgA tTG samples tested positive for IgA EMA. All samples with borderline IgA tTG results were negative for IgA EMA. Twenty-six percent (6/23) of the IgA deficient samples showed positive IgG DGP antibodies, but none of those tested positive for IgG EMA.

Conclusion : The seroprevalence of IgA tTG in this non-IgA deficient population (n = 1136) in Belgium is 1:114. The combined seroprevalence of IgA tTG and IgA EMA in that same population is 1:284. The seroprevalence based on positive IgA tTG or DPG IgG in, respectively, non-IgA deficient and IgA deficient subjects is 1:72 (n = 1159). These seroprevalences are similar to those found in neighbouring countries. (*Acta gastroenterol. belg.*, 2012, 75, 325-330).

Key words : Celiac disease, tissue transglutaminase antibodies, seroprevalence, IgA deficiency, children, Belgium.

Introduction

Celiac Disease (CD) is an immune-mediated enteropathy caused by the ingestion of gluten-containing cereals (wheat, rye, barley and possibly oats) in genetically susceptible individuals. The major predisposing genes are the histocompatibility leucocyte antigen (HLA)-DQ2 and DQ8 genotypes found in at least 95% of patients. CD is one of the most common lifelong disorders on a worldwide scale affecting 0.5-1% of the general population in developed countries. The disease is characterized by an invasion of the mucosal epithelium by lymphocytes, villous atrophy and crypt hyperplasia in the small intestine and by either a malabsorption syndrome (diarrhoea, steatorrhoea and weight loss) or minor apparently unrelated non-gastrointestinal symptoms such as iron-deficiency anaemia, arthralgia, infertility. Over the last twenty years, serology has changed the scope on CD

leading to the concept of the celiac iceberg, with asymptomatic, atypical or subclinical cases of celiac disease (1). Epidemiologic studies including all forms of celiac disease indicate that the prevalence in the majority of analyzed populations is similar, both among children and adults (2-6).

Currently anti-endomysium and human tissue transglutaminase IgA antibodies are considered the most sensitive and specific tests (7). In case of IgA deficiency, deamidated gliadin IgG antibodies have to be determined.

Prevalence data of CD in Belgium are not available. The aim of this study was to assess the seroprevalence of celiac disease in apparently healthy children and adolescents, using IgA tTG antibodies as an initial screening assay.

Materials and Methods

Population and design of the study

Residual sera from children and adolescents (1-19 years of age) were prospectively collected in 2006 by diagnostic laboratories. Only people living in Belgium were included. To obtain geographically well distributed samples, 15 diagnostic laboratories were involved that were spread over the 10 Belgian provinces. They were allocated fixed numbers of samples per age group to enable collection proportionally to the population of each region (Flanders, Wallonia, Brussels capital region) in the first place, and to the population of each province in the second. To avoid selection of immunosuppressed subjects, specific selection criteria were communicated to the collecting laboratories. Samples preferably had to be collected from emergency, otorhinolaryngology, and surgery/orthopaedic wards. Excluded were samples from oncology or intensive care wards as well as samples for which information about an immunosuppressed condition or multiple transfusions was available. The number of samples was stratified by age and gender, with equal numbers of males and females in each age band. Sera were stored frozen (-20° C) and delivered anonymously to the central laboratory: only birth date, sample date,

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gender and postal code of the place of residence were provided. The protocol was approved by the Ethics Committee of the University of Antwerp, conditional on the samples being delivered unlinked and anonymous to the investigators.

All samples were tested for total IgA levels and human tissue transglutaminase IgA antibodies (IgA tTG). In case of IgA deficiency or positive IgA tTG antibodies, IgA and IgG anti-endomysium antibodies (IgA/IgG EMA) and IgG antibodies against deamidated gliadin peptides (IgG DGP) were assessed.

Serum antibody tests

IgA tTG antibodies were detected by a commercial ELISA test (Zenit Celiac tTG IgA, Menarini Diagnostics), using a recombinant human tissue transglutaminase, in accordance with the manufacturer's instructions. Serum samples diluted to 1/51, calibrators and controls were incubated for 30 min in micro-wells at room temperature, then washed four times and subsequently incubated for 30 min with alkaline phosphatase-conjugated anti-human IgA. After a second series of washing, the reaction was revealed by the chromogenic substrate p-nitrophenyl phosphate (incubation for 30 min), and then stopped by adding stop solution. Antibody levels were expressed in ELISA units/mL using a standard titration curve. The cutoff value was set at 25 EU/mL. Samples with values between 20 and 25 EU/mL were considered borderline.

IgA/IgG EMA antibodies were tested with indirect immunofluorescence using commercially available fixed sections of monkey oesophagus (SCIMEDX). Sera were diluted to 1/5 in phosphate-buffered saline. EMA were detected with fluorescein isothiocyanate-labeled anti-human IgA or IgG conjugate (SCIMEDX). The intensity of the fluorescence was reported semiquantitatively (negative, 1+, 2+, 3+ or 4+). The slides were independently interpreted by two observers; the agreement rate was 100%.

IgG DPG antibodies were detected by a commercial ELISA test (Zenit Celiac Gliadin+ IgG, Menarini Diagnostics), using deamidated gliadin peptides, in accordance with the manufacturer's instructions. Serum samples diluted to 1/101, calibrators and controls were incubated for 30 min in micro-wells at room temperature, then washed four times and subsequently incubated for 30 min with alkaline horseradish peroxidase-conjugated anti-human IgG. After a second series of washing, the reaction was revealed by the chromogenic substrate tetramethylbenzidine (incubation for 30 min), and then stopped by adding stop solution. Antibody levels were expressed in ELISA units/mL using a standard titration curve. The cutoff value was set at 25 EU/mL. Samples with values between 20 and 25 EU/mL were considered borderline.

For IgA deficiency screening, all sera were tested by immunonephelometry on the BN Prospec nephelometer

(Siemens). Serum levels below the lower limit of normal for the appropriate age were considered indicative of IgA deficiency (8).

Statistics

Exact binomial 95% confidence intervals were calculated for the observed occurrences of positive results.

Results

A total of 1159 samples (603 male and 557 female) were analyzed in September 2009.

Total IgA levels below the lower limit of normal was detected in 23 children (1.98%) (95% CI 1.3-3%). Six of these children (1:4) (95% CI 1:2-1:10) had positive IgG DPG antibodies, but none of them tested positive for IgG EMA (Table 3).

Ten (0.86%) (95% CI 0.4-1.6%) had positive IgA tTG antibodies (group 1). A further 0.86% (n = 10) of the samples showed borderline results (group 2). IgA EMAs and IgG DPGs were determined for all borderline and positive tTG IgA samples, except for 4 of them because of insufficient residual sample volume (Fig. 1). In group 1, 4/8 (95% CI 15.7-84.3%) had positive IgA EMA's. Positive IgG DGP's were also obtained in half of the samples (4/8) with levels of more than 50 EU/mL. Three out of the 8 samples (95% CI 8.5-75%) were positive for both the IgG DPG and IgA EMA (Table 1). In group 2, all samples were negative for EMA IgA and 2 samples were positive for DPG IgG (Table 2).

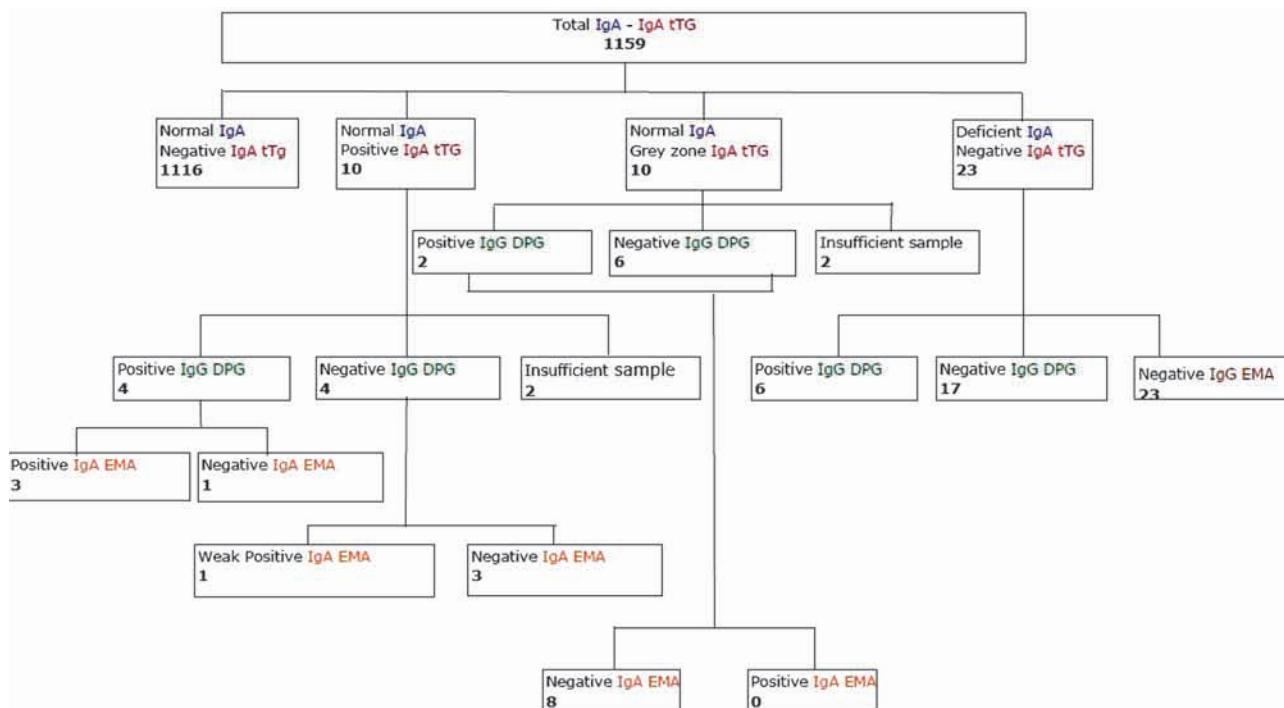
The seroprevalence of CD in the non-IgA deficient population based on a positive IgA tTG screening is 1:114 (95% CI 1:62-1:250). Only in half of these positive IgA tTG samples IgA EMA antibodies were detected. The seroprevalence of CD based on a positive IgA tTG and IgA EMA is therefore lower (1:284) (95% CI 1:111-1:1000).

The seroprevalence is even higher (1:72) (95% CI 1:45-1:125) when the 6 IgA deficient children with positive IgG DPG antibodies are considered as having CD.

Discussion

This is the first epidemiological study on the seroprevalence of CD in Belgium. The study was carried out on residual sera of children and adolescents without known chronic disorders living in different areas of Belgium.

The screening strategy was based on the measurement of total IgA and IgA tTG antibodies. In case of IgA deficiency, IgG antibodies were determined (9). Specificity of IgA tTG assays ranges between 95% and 99% (7,10) in both children and adults, with a better performance when human recombinant antigens are used (11-16). Van Meensel *et al.* (17) demonstrated a sensitivity and specificity of both 96% for the IgA tTG assay used in this study. Specificity seems to be better in research laboratories compared to clinical practice setting (18). Because



IgA tTG, tissue transglutaminase IgA antibodies ; IgG DPG, IgG antibodies against deamidated gliadin peptides ; IgA/IgG EMA, IgA/IgG anti-endomysium antibodies.

Fig. 1. — Results of screening autoantibodies associated with celiac disease in a population of Belgian children and adolescents (1-19 y).

Table 1. — EMA and DPG antibody results for all samples with positive IgA tTG results (group 1)

n°	Age	Sex	IgA tTG (EU/ml)	IgG DPG (EU/ml)	IgA EMA	IgG EMA
1	6	M	32,7/35,9	9,8	pos (1+)	neg
2	9	M	26,9/ins	ins	neg	neg
3	17	M	34,3/36,7	2,3	neg	neg
4	19	F	34,6/29,3	17,8	neg	neg
5	7	M	> 160,0/> 160,0	84 ^p	pos (4+)	pos (2+)
6	8	F	67,5/78,5	54,9 ^p	pos (2+)	neg
7	4	F	44,3/ins	ins	ins	ins
8	19	M	31,6/27,6	7,1	neg	neg
9	1	M	36,7/42,8	141,2 ^p	neg	neg
10	3	M	> 160/> 160	> 160 ^p	pos (4+)	pos (2+)

Ins : insufficient sample ; ^p = positive result for IgG DPG ; neg = negative anti-endomysium antibodies ; pos = positive result for anti-endomysium antibodies (semi-quantitative result).

of a higher positive predictive value of the EMA as compared to IgA tTG especially in the young infant, IgA and IgG EMA were also determined in samples with borderline and positive IgA tTG results (13,15,16,19-22).

In the present study the seroprevalence of CD in the non-IgA deficient population based on a positive IgA tTG screening was 1:114. If only the cases with combined positive IgA tTG and IgA EMA antibodies were taken into account, the seroprevalence of CD would be lower (1:284). In this study the diagnostic sensitivity and specificity of both assays could not be compared to the

golden standard because of the lack of histological confirmation of the diagnosis. The true seroprevalence is probably between 1:284 and 1:114 which is similar to what has been reported for the surrounding countries.

Based on a number of studies, the prevalence of CD worldwide is approximately 1:400 to 1:70 in the general population (1,23).

The discordance between the IgA tTG and the IgA EMA results can have several causes. The IgA tTG assay could lack specificity because of a too low cut off value for a positive result. Samples with markedly positive

Table 2. — EMA and DPG antibody results for all samples with borderline IgA tTG results (group 2)

n°	Age	Sex	IgA tTG (EU/ml)	IgG DPG (EU/ml)	IgA EMA	IgG EMA
1	15	F	22,3/18,6	< 1.0	neg	neg
2	17	M	22,7/14,1	6,2	neg	neg
3	4	M	22,1/< 1,0	30,1 ^p	neg	neg
4	13	F	22,8/25,4	ins	ins	ins
5	6	M	26,1/20,3	1,3	neg	neg
6	9	M	29/15,9	ins	ins	ins
7	5	F	20,3/13,8	26,1 ^p	neg	neg
8	17	F	23,3/19,5	9,8	neg	neg
9	9	F	25,9/20,3	5,8	neg	neg
10	6	M	21,5/17,9	10,8	neg	neg

Ins : insufficient sample ; ^p = positive result for IgG DPG ; neg = negative anti-endomysium antibodies.

Table 3. — EMA and DPG antibody results for all samples with IgA deficiency

n°	Age	Sex	IgA tTG (EU/ml)	IgG DPG (EU/ml)	IgG EMA
1	8	F	< 1,0	8,2	neg
2	9	F	< 1,0	40,3 ^p	neg
3	13	M	< 1,0	3,1	neg
4	15	F	< 1,0	126 ^p	neg
5	17	F	< 1,0	5,7	neg
6	19	M	2	< 1.0	neg
7	4	F	< 1,0	3,4	neg
8	15	F	1,7	4,2	neg
9	9	M	< 1,0	40,5 ^p	neg
10	1	M	< 1,0	1,5	neg
11	19	M	< 1,0	< 1.0	neg
12	9	M	< 1,0	15,7	neg
13	8	M	< 1,0	25,3 ^p	neg
14	15	F	< 1,0	8,2	neg
15	1	M	< 1,0	35,4 ^p	neg
16	3	M	<1,0	7,5	neg
17	7	F	< 1,0	10,1	neg
18	7	M	< 1,0	66,1 ^p	neg
19	17	M	< 1,0	< 1.0	neg
20	17	F	< 1,0	16,1	neg
21	1	F	< 1,0	2	neg
22	2	F	1,1	4	neg
23	18	F	1,6	9,6	neg

Ins : insufficient sample ; ^p = positive result for IgG DPG ; neg = negative anti-endomysium antibodies.

results (n = 3) for IgA EMA (2+ or more) had higher tTG antibody titers. The titer of 2 samples was higher than 160 EU/mL and the other sample had a titer of 75 EU/mL. The samples with negative IgA EMA results had IgA tTG antibody titers between 30-40 EU/ml. Increasing the cut-off value for positivity would perhaps improve specificity. This is supported by the fact that all

borderline IgA tTG samples showed negative IgA EMA's. Ben Hariz *et al.* (6) demonstrated that all individuals who were positive for IgA tTG but negative for IgA EMA had normal intestinal biopsies. The lower specificity for IgA tTGs compared to IgA EMA emerged from several other studies (13,15,16,20-22,24). A majority of these studies were performed in populations

of high CD prevalence. For that reason, the positive predictive value of this test is likely to be lower than reported when it is applied in a general population screening.

Another explanation could be a lower sensitivity of the used EMA assay. IgA EMA assays were shown to have a higher specificity but lower sensitivity compared to IgA tTG assays (15,20-22,25). The subjectivity in interpretation of the immunofluorescence could have resulted in more false negative results, although all slides were interpreted by 2 independent observers.

Selective IgA deficiency (SIgAD) occurs more commonly in patients with CD than in the general population (26,27). In these patients, CD will be missed when using the IgA tTG test (or any other IgA-based test, *e.g.*, IgA EMA) for screening purposes. Therefore IgA deficiency should be excluded in all patients suspected of CD. In SIgAD cases with CD, only IgG-type antibodies may be detected. The high diagnostic accuracy of the IgG class of DPG makes them a potential tool for diagnosis of CD in IgA-deficient patients. Recently, 3 studies described IgA-deficient CD children who all had low concentrations of IgA tTG and IgA DPG but high concentrations of IgG DPG (28-30). Villalta *et al.* (31) demonstrated that the IgG DPG test has high diagnostic sensitivity not only in IgA-competent but also in IgA-deficient CD patients. This is in contrast with the IgG tTG assay, which is specific for CD only in IgA deficiency (28).

In this study, we chose the IgG DPG assay to screen all IgA deficient samples. We also performed IgG EMA testing in those cases. Six of the 23 (1:4) IgA deficient samples were IgG DPG positive, with one result just above the manufacturer's cut-off. In none of these sample IgG EMA's could be detected. This is probably due to the lower sensitivity of IgG EMA assays compared to IgG DPG in IgA deficient patients (31).

The major drawback of this study is the lack of histological confirmation of the diagnosis. The validity of our findings is therefore dependent on the specificity and sensitivity of the serological assays that were used. The seroprevalence based on either positive IgA tTG or both positive IgA tTG and IgA EMA is comparable to what is found in seroprevalence studies with a similar design in other western countries.

In conclusion, we found a high seroprevalence of celiac disease in a population of Belgian children and adolescents, similar to what was found in the surrounding countries. A prospective study in Belgium performed from 1995 until 1997 showed a estimated incidence of CD in children is between 1:4000 and 1:5200 (32). Our study suggests that CD is underdiagnosed. Clinicians should keep the complex picture of the disease in mind and have a high index of suspicion with a low threshold for ordering serologic tests. A histological diagnosis still plays an important role in the final diagnosis of CD, although new algorithms propose to omit histological confirmation in case of a clear clinical picture and high titers of IgA tTG (33,34).

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