Symptoms and mucosal changes stable during rapid increase of pediatric celiac disease in Norway


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Abbreviations
CD, celiac disease; DGP, deamidated gliadin peptides; EMA, endomysial antibody; HLA, human leukocyte antigen; IELs, intraepithelial lymphocytes; Ig, immunoglobulin; tTG, tissue transglutaminase.

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ABSTRACT

Objectives: We aimed to study whether the incidence of pediatric celiac disease (CD) in South-Eastern Norway changed from 2000-2010. We also examined if there was a change in symptoms and histopathological morphology in the duodenal biopsies during the same period.

Methods: In three hospitals in South-Eastern Norway, records from pediatric patients (0-14.9 years) diagnosed with CD during two three-year periods (2000-2002 and 2008-2010) were reviewed. Only cases with a duodenal biopsy diagnosis of CD classified, as Marsh grade 2 and 3a-c were included. Frequencies of symptoms, anthropometric data and laboratory results were compared, in addition to re-examinations of histological sections from one of the hospitals.
**Results:** A total number of 400 cases were diagnosed with a female: male ratio of 1.5:1. The incidence rate for 2000-2002 was 15.9 cases per 100 000 person-years (95% CI 12.8-19.4), compared to 45.5 cases per 100 000 person-years during 2008-2010 (95% CI 40.5-50.9), p<0.001. The relative frequencies of symptoms and the distribution of histopathological changes were similar in the two periods, whereas weight z-scores and hemoglobin levels were significantly lower in the first period.

**Conclusions:** We found a three-fold increase in the incidence rate for CD in the Norwegian pediatric population during the decade 2000-2010. Slightly higher weight and hemoglobin levels at diagnosis in the latter period may be due to improved CD awareness. However, unaltered relative frequencies of symptoms and histopathological changes in the gut suggest a true increase of CD in Norwegian children.

**Keywords:** Celiac disease, child, epidemiology, symptoms, histopathology.

**What is known**
- Scandinavia has a relatively high incidence of pediatric celiac disease (CD).
- The incidence rates may be influenced by changed awareness of the disease.

**What is new**
- The incidence of pediatric CD patients in South-Eastern Norway has increased by three-fold during the decade 2000-2010.
- The severity of symptoms, age at diagnosis and histopathological changes were similar during the period.
- Abdominal pain, diarrhea and fatigue were the most common symptoms.
INTRODUCTION

Celiac disease (CD) is a chronic inflammation in the proximal small intestine caused by an inappropriate immune response against gluten proteins contained in wheat, barley and rye. There is a strong association between CD and certain gene variants within the human leukocyte antigen (HLA) complex, especially HLA-DQ2.5 and HLA-DQ8 (1). Patients may present a wide spectrum of clinical manifestations ranging from asymptomatic cases to very rare cases of celiac crisis with life-threatening severe diarrhea and multiple metabolic disturbances (2-4). More common symptoms and signs are diarrhea, abdominal discomfort or pain, dyspepsia, anemia, tiredness and growth failure, however constipation may also occur. In addition, young patients may develop dental enamel defects, joint pain, behavioral problems, lactose intolerance and epilepsy (5). Increased number of intraepithelial lymphocytes (IELs), crypt hyperplasia and villous blunting are the characteristic histopathology of CD in the proximal small intestine. In addition, there is an accumulation of leukocytes in the lamina propria, especially plasma cells, lymphocytes and histiocytes. Since 1969, when diagnostic guidelines of pediatric CD were established by the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN), examination of duodenal biopsies has been the gold standard for diagnosis until recently (6-8). Inflammation and changes in the duodenal architecture can be graded by a modified Marsh classification (9). In addition, blood samples positive for immunoglobulin A (IgA) endomysial antibody (EMA) and/or high titers of IgA antibodies against tissue transglutaminase (tTG) have become important for the diagnosis of CD. The presence of HLA-DQ2.5 and HLA-DQ8 can be used to support the diagnosis. Most patients improve on a gluten-free diet with the disappearance of symptoms and normalization of the architecture of the duodenal mucosa.

Previously regarded as a rare disease among young children, it is now known that CD is a relatively common disease. Approximately 1-2% of Caucasians have CD, and in some
populations in Scandinavia and Europe, the occurrence has increased dramatically in recent decades (10-16). The aim of this work was to assess whether there has been a change in the incidence of pediatric CD in South-Eastern Norway. As changes may take place due to increased awareness of the disease, we aimed to identify if there have been any changes in the severity of the clinical presentation and mucosal changes at diagnosis.

METHODS
Records from pediatric patients (0-14.9 years) subjected to upper gastrointestinal endoscopy for any reason were reviewed from three hospitals in South-Eastern Norway. During the study period, adolescents above this age received care from adult gastroenterology services. The three hospitals – Akershus University Hospital (Ahus), Oslo University Hospital (OUS)-Ullevål and Østfold Hospital Trust (SØ) – provide clinical services to approximately 23% of the total pediatric population in Norway. All pediatric endoscopies in this area are performed at these three public hospitals without any referral to other units, providing the opportunity to assess incidence rates by new cases detected at the hospitals. Two three-year periods, 2000-2002 and 2008-2010, were reviewed and compared to previously published incidence rates. Only cases with a duodenal biopsy classified as Marsh grade 2 and 3a-c, in a setting where other causes were considered unlikely, were classified as CD. The number of inhabitants aged 0-14.9 years in the catchment area was obtained from Statistics Norway. Symptoms, weight, height and blood chemistry parameters at the time of diagnosis were registered and compared. Histological sections from one of the hospitals (Ahus) were re-examined by two pathologists (A-C.R.B. and S.N.A.) blinded to identity, previously diagnostic result and time-period, to assess changes in severity of mucosal pathology. The Marsh-Oberhuber classification 2 and 3a-c was applied systematically to evaluate sections from the duodenal biopsies, and the biopsy diagnosis was obtained by evaluating well-orientated parts of the biopsy with the most
severely diseased areas (9, 17). The re-examination of all the duodenal biopsies by two pathologists was performed to ensure comparable, unbiased results from the two three-year periods.

This work was a part of an internal quality control to ensure that the patients had been correctly diagnosed with CD. The internal quality control was approved by the data protection officer in each of the three hospitals, and all data were anonymized before they were merged and analyzed.

**Data Analysis**

Chi-squared test or Fisher test were used to compare the incidence rates, symptoms, blood biochemistry and the Marsh grading in 2000-2002 and 2008-2010, SPSS 20.0 statistical software package (IBM SPSS inc., Chicago, IL, USA) and OpenEpi (http://www.openepi.com/Menu/OE_Menu.htm) were used for statistical analysis. P<0.05 was considered to be statistical significant.

**RESULTS**

In South-Eastern Norway we identified 96 pediatric patients (0-14.9 years) diagnosed with CD in 2000-2002 and 304 in 2008-2010, with a female: male ratio of 1.5:1 (Table 1). The incidence rate for 2000-2002 was 15.9 cases per 100 000 person-years (95% CI 12.9-19.4), whereas in the period 2008-2010 the incidence rate was considerably higher, 45.5 cases per 100 000 person-years (95% CI 40.5-50.9), p<0.001 (Figure 1). The mean age at diagnosis was 7.0 years (median 6.3, range 1.0-14.9) in 2000-2002 and 7.3 years (median 7.1, range 0.9-14.8) in 2008-2010, and for the two periods combined 6.5% were diagnosed before the age of two.
Weight z-scores were significantly lower in the first period (p<0.004) (Table 1). However, height z-scores were not significantly different in the two periods.

The relative frequency of symptoms in the two periods was not significantly different, with the most prevalent symptoms being abdominal pain, diarrhea and fatigue (Table 1 and Figure 2). The proportion of asymptomatic individuals with CD was few and not significantly different in the two periods. Most of the asymptomatic patients were screened for CD because of associated diseases. Diabetes mellitus was the most common associated disease. Thyroid disease and Down syndrome were other associated conditions, with no cases in the first period and only a small number of cases registered in the latter period. There was no family screening program at any time during the period, but the data on 1st or 2nd degree relatives indicates a non-significant increase in the proportion of cases with family risk of CD (Table 1).

Furthermore, the number of individuals with positive IgA EMA or elevated levels of IgA antibodies against tTG were not significantly different in the two periods (Table 1 and Figure 2). Before going through endoscopy, the children were examined for IgA EMA or/and IgA antibodies against tTG. Only four children had no results from these blood tests, either because they were not performed or because the information was not available in the medical records. In the first period 94.6% of the celiac cases tested positive for IgA EMA or/and IgA antibodies against tTG, whereas 96.7% tested positive in the latter period. Amongst individuals with negative IgA EMA or IgA antibodies against tTG (n=16 for the duration of the study) five were younger than two years old, five had family members with CD, and four had IgA deficiency. Moreover, three of these children had increased IgG antibodies against tTG, four had increased anti-gliadin IgA/IgG, one had increased IgA antibodies against deamidated gliadin peptides (DGP), and one had increased gamma/delta positive IELs (Marsh
3b) (18). Amongst those four with IgA deficiency, three were positive for IgG antibodies against tTG, while the other had increased anti-gliadin IgG.

Hemoglobin values were slightly lower in the first period (Table 1 and Figure 2).

Unfortunately, iron status was only available for a minority and could not be systematically reported.

The distribution of histopathological changes was similar in the two periods, and most of the patients were graded as Marsh 3c (Figure 3).

**DISCUSSION**

During the two three-years periods 2000-2002 and 2008-2010 the incidence rate for CD in the Norwegian pediatric population increased by three-fold. Relative frequencies of symptoms and histopathological changes in the small intestine were unaltered, whereas weight and hemoglobin levels were significantly lower in the first period compared with the latter.

The prevalence of CD in children (1-15 years old) in Telemark in South Norway during the period 1963-1978 was 1/900 (19), whereas in Hordaland in Western Norway during the two periods 1975-1989 and 1988-1991 it was 1/1706 and 1/625, respectively (20). In a recent study based on nation-wide data from the Norwegian Patient Registry, the prevalence was estimated to be 1/263 among 0-12 year old children (21).

During the period 1993-1999 the incidence rate in Akershus in South-Eastern Norway has been reported to be as high as 16.9 cases per 100 000 person-years (22). Akershus is part of the area examined in our work, and the result in Perminow’s study is comparable with the incidence rate found in the first study period (15.9 cases per 100 000 person-years) (Figure 1).

Furthermore, the high incidence rate of pediatric CD in Norway is similar to the high rates of CD registered in Scandinavia and Europe, especially Sweden and Finland (11-14, 16). Given
a stable incidence rate in our work we expect that 0.64% of children aged 15 years would be diagnosed with CD.

Improved public and professional awareness and better diagnostic tools may contribute in identifying cases of CD that previously went undiagnosed. Therefore, we aimed to study whether the increased incidence was accompanied by reduced severity of symptoms and mucosal changes. Lower weight and hemoglobin levels in the first period compared with the latter may suggest an increased awareness to CD in the latter period. However, in parts of the same period (1999-2000 to 2005) the mean weight among Norwegian 9-year-olds increased by the same magnitude as we found for children with CD (23). Frequencies of symptoms were similar during the study, and histopathological changes in the duodenal biopsies showed no statistical difference in the two three-year periods, indicating that CD was not diagnosed in a less severe grade or earlier stage in the latter period as a possible cause to an increase.

Additionally, the blood tests measuring IgA EMA and IgA antibodies against tTG, have been utilized for several years. Improved sensitivity of the antibody assays cannot be ruled out, however we find it unlikely that better diagnostic tools should explain the rapid increase in diagnosed CD.

Lately, a true increase in CD has been demonstrated by screening studies in adults where awareness or better detection rate cannot be attributed (15, 24). Genetic (HLA-DQ2.5/DQ8) and environmental (gluten) factors that cause CD are therefore remaining factors of importance. Genetic factors change extremely slowly and are therefore unlikely to contribute significantly to the rapid increase of CD within the study period, whereas environmental factors like changes in diet, gut microbiota, hygienic and economic status might play an important role (25, 26). During the period of interest only minor changes in living conditions, hygiene and vaccination (pneumococcal vaccine introduced in 2006) policy have occurred. Gluten is believed to be the most essential environmental element in the development of CD,
and the change in gluten consumption in the society is consequently of great interest (27).
However, the role of quality and quantity of gluten as well as timing of gluten introduction
with or without ongoing breast-feeding is debated. In our work, we do not have information
on infant feeding practice for each case. However, the study covers a period in which new
guidelines for the nutrition of infants were implemented in Norway (2001), which include the
recommendation to delay the introduction of any weaning foods to after 6 months of age
(https://helsedirektoratet.no/Lists/Publikasjoner/Attachments/240/Anbefalinger-for-
spedbarndsernering-IS-1019.pdf). The timing of gluten introduction may be of importance,
with two observational studies suggesting lower risk with introduction of gluten in natural
amounts during four to six months of age as compared to later introduction (28, 29).
However, a recent randomized trial did not find any protection by small amounts of gluten
from four months of age (30). Furthermore, meta-analyses do not support that a specific
practice of gluten introduction and breastfeeding could explain the increase in incidence
observed in our work (31).
During the study period 2000-2010, the diagnostic criteria for CD in use were identical, and
prior to the change of diagnostic guidelines of pediatric CD established by the European
Society of Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) in 2012 (8). In
these new diagnostic guidelines, duodenal biopsies are no longer required for the diagnosis of
CD in all children. Diagnosis can be made by a blood sample when IgA antibodies against
tTG are at levels over ten times the upper limit of normal value, followed by a confirmation of
a positive IgA EMA test in a separate blood test. As earlier, positivity for HLA-DQ2.5 or
HLA-DQ8 strengthens the diagnosis. Importantly, our work has consistently applied the
ESPGAN guidelines from 1990 (7), and duodenal biopsies were uniformly taken from the
descending part of the duodenum, except in a small number of cases where biopsies were also
taken from the duodenal bulb in the latter period. A change in diagnostic practice should not impact our results.

In our work, patients with IgA deficiency were either positive for IgG antibodies against tTG or had increased anti-gliadin IgG. The novel guidelines from 2012 suggest IgG antibodies against DGP should be used as an additional test in individuals negative for other serological markers of CD and in children under the age of two years old. In our work, five children younger than two years old with biopsy-proven CD tested negative for IgA EMA and IgA antibodies against tTG, illustrating that these tests may have lower sensitivity in this age group. Unfortunately, IgG antibodies against DGP were not examined in any of these children. HLA status on these children would also have been of interest, but was not widely available for clinical purposes in our study period. Furthermore, a false positive diagnosis of CD should also be considered in children younger than two years old with negative serology, because other diseases like cow-milk allergy and parasites could cause mucosal changes indistinguishable from the changes seen in CD.

During the duration of our work the number of upper endoscopies for any indication increased by two-fold. However, the number of serological tests (EMA/tTG) performed for the population in the catchment area would probably be of more interest. The indication for gastroscopy was based on symptoms, blood chemistry parameters, associated diseases and/or family history, which means that all with suspected CD had to perform a duodenal biopsy. The fact that the number of asymptomatic cases of CD in children has not increased in this period, strengthens our assumption that increased diagnostic activity cannot entirely explain the increase of CD. A cross-sectional screening study would be required to confirm whether the incidence of pediatric CD in Norway is clearly increasing.

A limitation of this work is that it is a retrospective study. Some cases could be missed, the data is not complete, and consequently all questions of interest can not be answered. To
conclude, CD in Norwegian children has increased rapidly during the decade 2000-2010, consistent with increased incidence of CD in Scandinavia and Europe during the last decades. It is possible that, higher awareness and better diagnostic tools might lead to diagnosis of CD in an earlier stage with milder symptoms and minor histological changes in the duodenal mucosa. However, no significant alterations of symptomatology or mucosal histopathology in the compared periods of time were found in our work. This suggests a true rise in the incidence of CD. Although increased awareness and better diagnostic tools may contribute, further research is warranted to identify other possible factors causing this increased incidence of CD.

Acknowledgments

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REFERENCES


FIGURE LEGENDS

**Figure 1:** Incidence rates [cases per 100 000 person-years] for celiac disease in the Norwegian pediatric population in 2000-2002 and 2008-2010 compared with previously published incidence rate from the same area 1993-1998 (22). Statistical significance (p<0.05) is marked on the graph.
Figure 2: Symptoms and signs, anthropometric data and laboratory results at diagnosis of celiac disease in Norwegian children in 2000-2002 and 2008-2010. Statistical significance (p<0.05) is marked on the graph.
Figure 3: Histopathological changes in the duodenal mucosa graded by modified Marsh-Oberhuber classification grade 2 and 3a-c in the Norwegian pediatric population in 2000-2002 and 2008-2010: Marsh 2 has intraepithelial leukocytes (IELs)>30/100 enterocytes, crypt hyperplasia and normal villous width and length. Marsh 3 has IELs>30/100 enterocytes, crypt hyperplasia and wider villous with initial [3a], subtotal [3b] and total [3c] villous blunting (9).
### Table Legends

**Table 1:** Anthropometric data, symptoms and signs, associated diseases, family history and laboratory results at diagnosis of celiac disease in Norwegian children in 2000-2002 and 2008-2010. Statistical significance p<0.05 (*).

<table>
<thead>
<tr>
<th></th>
<th>2000-2002</th>
<th>2008-2010</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of cases</td>
<td>96</td>
<td>304</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Incidence rate (cases/100,000/year)</td>
<td>15.9</td>
<td>45.5</td>
<td></td>
</tr>
<tr>
<td>Mean age at diagnosis (median)</td>
<td>7.0 (6.3)</td>
<td>7.3 (7.1)</td>
<td>0.4</td>
</tr>
<tr>
<td>Age &lt; 2 years</td>
<td>10 (10.4)</td>
<td>16 (5.3)</td>
<td>0.07</td>
</tr>
<tr>
<td>Female gender (%)</td>
<td>58.3</td>
<td>60.2</td>
<td>0.75</td>
</tr>
<tr>
<td><strong>Height and weight SDS</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean height SDS all</td>
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<td>-0.13</td>
<td>0.16</td>
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<tr>
<td>Mean height girls</td>
<td>-0.25</td>
<td>-0.11</td>
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</tr>
<tr>
<td>Mean height boys</td>
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<td>Mean weight SDS all</td>
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<td>-0.08</td>
<td>0.01*</td>
</tr>
<tr>
<td>Mean weight girls</td>
<td>-0.36</td>
<td>-0.04</td>
<td>0.02*</td>
</tr>
<tr>
<td>Mean weight boys</td>
<td>-0.40</td>
<td>-0.14</td>
<td>0.27</td>
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<tr>
<td>Height &lt; -1 SDS</td>
<td>25</td>
<td>25</td>
<td>0.65</td>
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<td>Weight &lt; -1 SDS</td>
<td>30.8</td>
<td>16.8</td>
<td>0.004*</td>
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<tr>
<td><strong>Abdominal symptoms (%)</strong></td>
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<tr>
<td>Abdominal pain</td>
<td>59.4</td>
<td>67.8</td>
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<tr>
<td>Diarrhea</td>
<td>47.9</td>
<td>47.7</td>
<td>0.97</td>
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<tr>
<td>Constipation</td>
<td>16.7</td>
<td>18.8</td>
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<tr>
<td><strong>General symptoms and signs (%)</strong></td>
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<tr>
<td>Anorexia</td>
<td>19.8</td>
<td>11.8</td>
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<tr>
<td>Weakness</td>
<td>28.1</td>
<td>25.7</td>
<td>0.63</td>
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<td>Irritability</td>
<td>9.4</td>
<td>3.9</td>
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<tr>
<td>Pallor</td>
<td>11.5</td>
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<td>Growth failure (weight/length)</td>
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<td>23.7</td>
<td>0.002*</td>
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<td>Asymptomatic, n (%)</td>
<td>9 (9.4)</td>
<td>19 (6.3)</td>
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<td>Associated diseases, n (%)</td>
<td></td>
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<td>Down syndrome</td>
<td>16 (16.7)</td>
<td>6 (6.1)</td>
<td>0.003*</td>
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<tr>
<td>Diabetes</td>
<td>0</td>
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<td>Thyroid disease</td>
<td>16 (16.7)</td>
<td>14 (4.6)</td>
<td>&lt;0.001*</td>
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<td>Family history (1st or 2nd degree), n (%)</td>
<td>18 (18.9)</td>
<td>88 (28.9)</td>
<td>0.05</td>
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<td>Laboratory results at diagnosis</td>
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<tr>
<td>Mean hemoglobin (median)</td>
<td>11.9 (11.9)</td>
<td>12.2 (12.4)</td>
<td>0.04*</td>
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<td>Hemoglobin &lt; 11.0 g/dL (%)</td>
<td>16/75 (21.3)</td>
<td>36/252 (14.3)</td>
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<td>Elevated anti-tTG IgA/EMA, n (%)</td>
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<td>293 (96.7)</td>
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<td>IgA-deficiency (%)</td>
<td>1 (1.0)</td>
<td>3 (1.0)</td>
<td>0.88</td>
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