A Retrospective Application of the European Society for Paediatric Gastroenterology Hepatology and Nutrition Guidelines for Diagnosis of Coeliac Disease in New Zealand Children

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Abstract In 2012 the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) published an updated guideline for the diagnosis of Coeliac disease, allowing symptomatic individuals with appropriate serology and HLA genotype to be diagnosed without a small bowel biopsy. This retrospective study aimed to assess the applicability of this guideline to children in New Zealand. Children less than 16 years of age investigated for coeliac disease with small bowel biopsy in Christchurch Hospital between January 2010 and December 2012 were identified. The results of those with tissue transglutaminase IgA (tTG) levels greater than 50 units (10 times upper limit of normal), positive endomysial antibodies and HLA DQ2/DQ8 were used to calculate sensitivity and specificity. Data from 160 children was available: 70 had biopsy-confirmed Coeliac disease, and 90 had negative biopsies. Limited data precluded application of the guidelines to all patients. Using only tTG data, levels above 50 units provided a sensitivity of 67% and a specificity of 92%. Specificity increased to 97% when limited EMA and HLA DQ2/DQ8 data was added. Despite limited data, applying the ESPGHAN guidelines in the Christchurch paediatric population over this period produced a high specificity (97%). Prospective studies are now required to confirm these findings.

Keywords: ESPGHAN, tissue transglutaminase, endomysial antibodies, HLA-DQ2/DQ8, children

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1. Introduction

Coeliac disease (CD) is a systemic immune-mediated disorder triggered by dietary gluten in genetically susceptible individuals. [1] CD may present with a variety of gastrointestinal or extraintestinal symptoms. These may include diarrhoea, constipation, abdominal pain, nausea, vomiting, weight loss, failure to thrive, delayed puberty, iron deficiency anaemia, irritability, and fatigue. [2] Greater appreciation of the wide spectrum of presenting features of CD has led to increased focus upon this diagnosis. General awareness in the community about CD is also increasing with many restaurants [3] and supermarkets increasing their range of gluten free options. Coincident with this is increased parental awareness of the possibility of CD in their child.

Over recent years, the diagnosis of CD has been based upon the histological assessment of mucosal biopsies obtained endoscopically from the proximal small bowel. Although this is seen as the gold standard, this investigation is relatively invasive, is not 100% sensitive,

and may lead to some delay in confirmation of the diagnosis. In 2012 the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) reviewed the diagnostic guidelines for CD and proposed a non-biopsy based diagnostic approach for a proportion of symptomatic children. [2] This guideline proposed that symptomatic children with a highly positive tTG IgA level (at least ten times the upper limit of normal (ULN)), positive endomysial antibodies (on a separate occasion), and an appropriate at risk genotype (HLA DQ2 or DQ8), could be diagnosed with CD without undergoing a small bowel biopsy (SBB). This new approach excluded specific groups of children, such as asymptomatic children in high risk groups (including those with a first degree relative with CD or those with Type 1 Diabetes mellitus). According to the ESPGHAN guideline, children in these categories should still undergo a traditional assessment with SBB as the defining investigation.

Recent data from New Zealand demonstrates increasing numbers of diagnoses of CD in children, [4] with one report indicating a prevalence of doctor-diagnosed CD in 1% of a group of young children. [5] To date the

ESPGHAN guidelines have not been utilised in the NZ setting. This study aimed to retrospectively apply the ESPGHAN diagnostic criteria to a group of children assessed in Christchurch, NZ.

2. Methods

2.1. Study Populations

The study population was children aged less than 16 years who had undergone endoscopic SBB for investigation of possible CD at Christchurch Hospital, Christchurch, NZ, in the three years between 2010 and 2012. The Anatomical Pathology Department Database was used to identify a group of subjects with SBB proven CD and a control group with normal SBB histology.

2.2. Data Collection

The clinical records for each subject were reviewed for information on demographic data (including age, ethnicity, and gender), growth parameters, symptoms, personal history, family history, and relevant investigations. Demographic data was obtained from the Hospital database. Growth concerns were recorded if mentioned in documentation, a child had lost weight, or had static weight recorded. Information was obtained from General Practitioner referral letters, outpatient and inpatient consultations, endoscopy reports, histology forms, electronic laboratory records, and the Anatomical Pathology Department Database.

2.3. Laboratory Data

Tissue transglutaminase IgA antibody levels were measured by Celikey IgA Varelisa enzyme-linked immunosorbant assay (Phadia AB, Uppsala, Sweden). The normal range for this assay was< 2units, equivocal 3-5units and elevated >5 units. Endomysial antibody levels were measured with the NOVA Lite immunofluorescence assay containing primate eosophagus and Monkey Absorbed IgA antibody (Inova Diagnostics, California, USA). This was reported as 'not detected', 'weak' or 'positive'. HLA DQ2 and DQ8 genotypes were measured by sequence specific polymerase chain reaction and reported as 'not detected' or 'positive for HLA-DQ2 and/or DQ8'. The diagnosis of CD was based upon formal histological assessment of the SBB by an Anatomical Pathologist, using features of the Marsh criteria.[6] The control group did not have histological features of CD. The normal ranges of other investigations (iron, ferritin, B12, folate and vitamin D) were as performed by Canterbury Health Laboratories (Christchurch, NZ).

2.4. Data Analysis

Statistical comparisons were done with Graphpad QuickCalcs software (GraphPad Software, Inc. La Jolla, CA, USA). [7] The categorical data were compared between groups using contingency table analysis with the χ^2 test or the Fisher's exact test, as appropriate. Continuous data were analysed using an unpaired t test. Weight and height z scores were calculated from growth data with the World Health Organisation percentile calculators and charts. [8,9] A p value of < 0.05 was considered significant.

3. Results

3.1. Study Population

Over the three year period from 2010-2012, 194 endoscopic SBB were performed on children under 16 years of age at Christchurch Hospital looking for a diagnosis of CD:34 of these children were excluded from analysis (Table 1). The remaining study population comprised 160 children; 70 with biopsy proven CD, and 90 with biopsies negative for celiac disease.

Table 1. Subjects under 16 years of age who had small bowel biopsies for investigation of coeliac disease were excluded

Description	Number
Follow up biopsies for CD	11
Inflammatory bowel disease	9
Investigation for other pathologies	6
Asymptomatic screening biopsies	
Type 1 Diabetics	4
Children with Down Syndrome	3
Children with Turners Syndrome	1
Total	34

3.2. Demographics

There was a higher ratio of females to males among the subjects with CD (1.9:1 versus 1.27:1 in those without; p=0.003). (Table 2). There were no differences between the two groups with regards to anthropometry, age, or ethnicity.

Table 2. Demographic characteristics of the study population

Demographic Data	Coeliacs	Controls	P value
Female	46 (66%)	40 (44%)	0.003
Mean age $\pm$SD	7y $\pm$3y 11m	7y 5m $\pm$4y 3m	0.56
Weight (kg) mean Z score $\pm$SD	0.14 $\pm$1.36	0.22 $\pm$1.43	0.75
Height (cm) mean Z score $\pm$SD	0.14 $\pm$1.05	0.21 $\pm$1.42	0.82
Ethnicity			
NZ European	61 (87%)	78 (87%)	1.0
Other European	6 (8.5%)	5 (5.5%)	
Other	1 (1.4%)	0	
Maori	1 (1.4%)	4 (4.4%)	
Tongan	1 (1.4%)	0	
Chinese	0	1 (1%)	
Fijian	0	1 (1%)	
Not Specified	0	1 (1%)	

3.3. Symptoms

Gastrointestinal symptoms were more common in the control group than in the children with CD (87% vs 69%: p=0.007). (Table 3). However, the presence of specific gastrointestinal symptoms or extraintestinal symptoms were similar in both groups (p>0.05 for all). Abnormal

examination findings were documented in only four patients: abdominal distension was noted in two children with CD and one control, whilst one child with CD was felt to have decreased muscle bulk.

Table 3. Symptoms in subjects with CD and Controls

Symptoms	Coeliac Disease (n=70)	Controls (n=90)	p value
Gastrointestinal	48 (69%)	78 (87%)	0.007
Vomiting	6 (9%)	16 (18%)	0.11
Abdominal pain	36 (51%)	54 (60%)	0.34
Restricted diet	7 (10%)	3 (3%)	0.11
Decreased appetite	7 (10%)	7 (8%)	0.78
Nausea	6 (9%)	11 (12%)	0.61
Change in bowel habit	25 (36%)	43 (48%)	0.15
Diarrhoea	22 (31%)	31 (34%)	0.74
Constipation	12 (17%)	16 (18%)	1.0
Growth concerns	12 (17%)	16 (18%)	1.0

3.4. Family History

Eighteen (26%) children with CD were noted to have a family history of CD: in 12 cases this was documented as a first degree relative. Similarly, 20 (22%) of the control group had a family history noted, with 9 documented as a first degree relative (p>0.05 compared to CD group).

3.5. Laboratory Data

More children with CD had an anti-tTG IgA antibody result documented than in the control group (87% vs 70%; p = 0.01). (Table 4) Furthermore, more children with CD had a tTG result that was ten or more fold greater than the normal range (67% vs 8%; p <0.001). The majority of children with CD with EMA testing were positive (95% vs 19%; p = <0001). Only five of the CD group had a HLA DQ2/DQ8 measured, and all were positive.(Table 4).Twenty-eight (31%) of the control group had an HLA DQ2/DQ8 performed: 19 were positive (68%).

Table 4. Blood results in subjects with CD and controls

Blood tests	Coeliac disease	Controls	p value
tTG Ig A antibodies			
Tested	61 (87%)	63 (70%)	0.01
5-49 units (positive)	13 (21%)	17 (27%)	0.53
> 50units	41 (67%)	5 (8%)	<0.001
Endomysial Antibodies			
Tested	41 (59%)	42 (47%)	0.15
Positive	39 (95%)	8 (19%)	<0.0001
HLA DQ2/DQ8			
Tested	5 (7%)	28 (31%)	0.0002
Positive	5 (100%)	19 (68%)	0.29
Full blood count			
Tested	43 (61%)	60 (67%)	0.51
Anaemia present	2 (5%)	5 (8%)	0.70
Nutrient deficiency			
Tested for	43 (61%)	51 (57%)	0.63
Present	17 (40%)	9 (18%)	0.02
Iron deficiency tested for	41 (59%)	50 (56%)	0.75
Iron deficiency	16 (39%)	5 (10%)	<0.002

The tTG 50 unit cut off (ten times the upper limit of the local normal range) gave a sensitivity of 67% (95th CI 54-79%), specificity of 92% (95th CI 82-97%), a positive predictive value (PPV) of 89% (95th CI 76-96%), and negative predictive value of 74% (95th CI 76-96%). Adding the limited EMA and HLA data increased the specificity to 97% (95th CI 89-99.5%), and PPV to 95% (95th CI 84-99%).

Specific micronutrient deficiencies were tested for in 61% of subjects with CD and 57% of those without (p >0.05).(Table 4). Iron levels were tested in 59% of those with CD and 56% of controls (p >0.05): iron deficiency was seen more commonly in the children with CD than in the control group (39% vs 10%; p <0.002).

4. Discussion

This limited retrospective application of the ESPGHAN diagnostic algorithm to symptomatic children in New Zealand, generated a moderately high specificity (97%) and PPV (95%). However, missing data points resulted in incomplete evaluation in this setting.

A number of prospective studies have recently evaluated the ESPGHAN guideline in different settings. A prospective study of 412 Italian adults and children with symptoms or signs consistent with CD, found a positive EMA combined with a tTG IgA ≥7 times the ULN resulted in a specificity and PPV of 100% for diagnosis of CD (mucosal changes ≥Marsh grade 2). [10] A prospective analysis of children undergoing investigation for CD in the Netherlands evaluated tTG IgA and EMA antibodies in 183 children.[11]All 87 of the patients with tTG >100 U/mL (10 times ULN) had CD on SBB; PPV and specificity 100%, and sensitivity 73% (95% CI 63-80%).

Similarly, Sandström et al [12] prospectively screened 7161 children in Sweden with tTG IgA, EMA, and HLA DQ2/DQ8 and diagnosed 153 children with CD on SBB. tTG values of ≥10 times ULN (50U/mL) with positive EMA and HLA-DQ2 or DQ8 had a PPV and specificity of 100%. A retrospective analysis of 150 children in Spain with tTG levels ≥ 10 times ULN, positive EMA andHLA-DQ2 or DQ8 found a positive predictive value of 97% initially but this increased to 100% after the three patients with 'false positive' results had a gluten challenge and repeat SBB [13] The sensitivity of these tests in this cohort was 82%. Despite the retrospective design of the current study, the specificity and PPV approach that seen in these prospective studies. This suggests that prospective analysis may confirm that the ESPGHAN diagnostic algorithm maybe applicable to the New Zealand paediatric population using local laboratory data.

It is important to avoid an incorrect diagnosis of CD, which would lead to unnecessary dietary restriction, extra family time and effort to prepare foods, and for some children potential growth impairment. Symptoms are likely to continue and the correct diagnosis may be missed. If this current study is representative of the local population, up to three children out of every hundred investigated for CD could be falsely diagnosed by adopting this guideline. The retrospective nature of this research resulted in only one child with CD and 19 controls having all three laboratory studies performed. The majority of children also had all coeliac serology performed concurrently (rather than sequentially), potentially increasing the risk of false positives. The higher specificities and positive predictive values seen in

recent prospective studies [11,12] highlight the importance of prospective analysis; ensuring the correct subjects enrolled into the correct groups, have the correct tests, at the correct times, to get the most accurate results from the ESPGHAN guidelines.

While the sensitivity of the ESPGHAN guideline in the current study was relatively low (67%) it was comparable to the sensitivity of 73% seen in the Dutch study, [11] but lower than the 82% in the Spanish study. [13] Increasing sensitivity would risk compromising the high specificity obtained. The aim of the ESPGHAN guideline is to decrease the number of children requiring a SBB for diagnosis rather than eliminating it from the diagnostic pathway. If 67% of CD diagnoses could be made without a small bowel biopsy in Christchurch, this would be a large saving in duration of symptoms, anaesthetic and procedural risk, and cost to the health system.

Many studies have looked at the sensitivity and specificity of CD antibodies. In this study, the tTG IgA antibody ELISA used had a sensitivity of 89% (95% CI:81-94%), and specificity of 65% (95% CI:55-74%). EMA had a sensitivity of 95% (95% CI:89-98%) and specificity of 81% (95% CI:72-88%). These sensitivities are lower than reported elsewhere. [14]This may reflect the relatively small number of children in our study, difference in laboratory testing kits, or accidental inclusion of children with other causes of intestinal inflammation due to retrospective collection of data. Some children with elevated tTG or EMA but normal SBB will subsequently develop CD [13,15]. Auricchio et al [16] followed a group of 175 asymptomatic children with elevated tTG for nine years; 43% continued to have elevated tTG, 20% became negative, and 37% fluctuated between elevated and normal. Overall 67% still had normal SBB after nine years of follow up.

Very few subjects in the current study underwent HLA genotyping. This probably reflects the recent introduction of this test and its absence from the current diagnostic algorithm. Far more controls had their HLA genotype tested, showing its usefulness as a test of exclusion in children with mildly elevated or equivocal tTG.

The ratio of males to females (1:1.9) in our CD group was slightly lower than those reported in paediatric populations in other studies, 1:2.7 in Spain, [17] and 1:2.5 in Auckland, New Zealand. [18] However it was higher than the ratio of 1:1.4 found in a previous local study by Cook et al [4] that included a small sub-group of children under 19 years old.

There was no difference between ethnicities in the two groups, however Maori were under represented. Nine percent of the Christchurch population identify as Maori [19]. This contrasts with just 1.4% in the CD group and 4.4% in the control group of the current study. This is consistent with the higher prevalence of CD in European populations, [20] however it does not explain why Maori children are under-represented in the control group. This may be due to lower clinical suspicion in this ethnic group, less access to health care, or the sample size. Ethnicity data was supplied by parents and recorded in the hospital electronic database. This database will only allow one ethnicity to be recorded for each patient. Mixed heritage is becoming increasingly common, so this data source may be inaccurate.

This study showed a higher frequency of gastrointestinal symptoms in children without coeliac disease (p=0.007). This may be due to a number of children with CD presenting with only extraintestinal symptoms. Previous studies have also failed to show increased gastrointestinal symptoms in those with CD. [21,22].

Multiple findings in our study suggest that the retrospective design limited the accuracy of data collection. Ten percent of children in both groups had limited documentation in their notes, some due to direct referral for SBB (high tTG levels) from local practitioners and peripheral centres. Fewer children in the control group had tTG measured and low numbers had all three assessments. Potential inaccuracies in identifying appropriate children for this study may have limited the ability to detect differences between the two groups, and decreased the apparent sensitivity of tTG measurement. The study included a moderate sample size, but this may have been inadequate to fully represent smaller population groups, or the smaller group of children with mainly extraintestinal CD symptoms.

5. Conclusion

Limited use of HLA DQ2/DQ8 testing limited the assessment of the ESPGHAN guidelines in the current population. However, the isolated use of the anti-tTG antibody cut off of 10 times the ULN, generated moderately high specificity of 92%. A potential 67% reduction in the number of diagnostic biopsies performed would significantly reduce the work load and cost to the health system along with inconvenience and stress to families. This would likely increase patient satisfaction with the diagnostic process. The value of the 2012 ESPGHAN Guideline for the diagnosis of CD in New Zealand should now be evaluated prospectively.

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