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Celiac disease in children with inflammatory bowel disease: a prospective cohort study

by

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Abstract

The aim of this work was to examine any possible IBD activity-related variations in immunoglobulin A (IgA) anti-tissue transglutaminase (tTG) levels in children with IBD. The prevalence of CD in children with IBD was also examined. In a prospective cohort study, children with IBD were screened for celiac disease using anti-tTG IgA antibodies and endoscopy performed if positive. Age-matched controls without IBD were recruited. One hundred and sixty four children were recruited in each arm of the study. There was no correlation between changes in IgA anti-tTG antibody titers and changes in disease activity indices. The prevalence of celiac disease among patients and controls was similar (1/164 (0.6%)). In children with IBD, changes in disease activity do not significantly affect serum levels of anti-tTG IgA antibodies. Anti-tTG IgA antibodies should not be used to monitor IBD activity. Children with IBD should not be routinely screened for CD.

Acknowledgement

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Abbreviation List

AGA: Antigliadin antibodies

CD: Celiac disease

CrD: Crohn's disease

DGP: Deamidated gliadin peptide

EGD: Esophagogastroduodenoscopy

ELISA: Enzyme-linked immunosorbent assay

EMA: Endomysial antibodies

IBD: Inflammatory bowel disease

IBS: Irritable bowel syndrome

Ig: Immunoglobulin

OR: Odds ratios

PCDAI: Pediatric Crohn's disease activity index

PUCAI: Pediatric ulcerative colitis activity index

tTG: Tissue transglutaminase

UC: Ulcerative colitis

Chapter 1 Introduction & literature review

Introduction:

Both celiac disease (CD) and inflammatory bowel disease (IBD) are immune-related disorders. These two disorders may be linked together (1). This thesis explores the possibility of a possible link between both disorders in the pediatric age group. Over the next few pages, a summary of the epidemiology, pathogenesis and methods of diagnoses of both disorders is provided.

Literature review

1.1. Celiac disease

Celiac disease (CD) is an immune-mediated disorder that affects primarily the gastrointestinal tract. It is characterized by small bowel inflammatory changes resulting in mucosal injury and subsequent malabsorption in genetically susceptible individuals following exposure to gluten (2). Gluten is a family of storage proteins found primarily in wheat (2).

1.1.1. Epidemiology

Before recognizing the link between gluten and CD, up to 12 % of children with CD were reported to die due to their disease (2). During the second World War, Dutch pediatricians noticed that patients with CD got better when they consumed less bread, a common event because of reduced food supplies. After the

war, properly controlled studies demonstrated the link between celiac disease and gluten-containing food (3). Other food elements like barley and rye were also proven to trigger the disease.

The major toxic element was found to be the alcohol-soluble gliadin fraction of wheat gluten (4).

Population-based studies, worldwide, estimate that the prevalence of CD ranges between 1:80-1:150 (5, 6). About 15% of first-degree relatives to patients with CD will have celiac disease (7) and, amongst monozygotic twins, the concordance rate is up to 75% (8).

1.1.2. Pathogenesis

Gluten is a family of storage proteins found in wheat. It can be separated into ethanol-insoluble glutenins and alcohol-soluble gliadins. Proteins similar to gliadins can be found in rye, barley and oats (9). Upon exposure to these proteins in genetically susceptible individuals, an inflammatory response will be triggered that ultimately leads to adverse changes in small bowel structure and function. Between 85% and 95 % of patients with CD carry the HLA class II gene HLA-DQ2. The remaining patients with CD carry the HLA class II gene HLA-DQ8. These molecules are normally expressed in antigen-presenting cells (APC). Upon exposure to gluten proteins, activation of CD4^{+} helper (Th1) cells in the lamina propria of the intestine takes place, resulting in crypt hyperplasia and villous atrophy (7). HLA-DQ2 or HLA-DQ8 loci are expressed in 30-35% of the populations where celiac disease is prevalent, but only 2%-5% of gene carriers

develop CD. This suggests that other non-genetic e.g. environmental factors are involved in the development of symptoms of CD. Consequently, individuals who are genetically susceptible to CD may not develop symptoms unless they are exposed to environmental factors (10). Such environmental factors include early massive exposure to gluten (11), early gastrointestinal infections (12) or changes of gastrointestinal bacterial flora (13).

Almost all patients with CD develop immunoglobulin A (IgA) antibodies to the enzyme tissue transglutaminase (tTG) which is expressed by many cell types (14). When cells are under mechanical or inflammatory stress, tTG is released into the extracellular space (15,16). Tissue transglutaminase transforms intracellular neutral glutamine to negatively charged glutamic acid residues (17). These residues will bind to HLA-DQ2 or HLA-DQ8 loci and trigger an inflammatory T-cell response.

1.1.3. Diagnosis

The typical presenting symptoms of CD include chronic diarrhea, abdominal pain and faltering growth. However, patients with celiac disease can be asymptomatic (18). The North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) guidelines recommend testing for CD in children with failure to thrive, persistent diarrhea, chronic constipation, recurrent abdominal pain or vomiting, dental enamel hypoplasia of permanent teeth, idiopathic short stature, significant pubertal delay and chronic iron deficiency anemia unresponsive to iron supplementation (18). Serologic tests for celiac

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disease are mainly used for initial screening for the disease. However, the gold standard for diagnosis is the presence of the typical histopathological changes in small bowel biopsies obtained via esophagogastroduodenoscopy (EGD) (18).

1.1.3.1. Serological screening

The first serologic tests for CD were developed in the early 1980s and measured IgG and circulating IgA anti-gliadin antibodies (AGA). These tests have diagnostic sensitivities of 82% to 89% and specificities of 66% to 90% respectively (19). However, the positive predictive value of AGA testing in most populations is less than 30%, thus AGA testing has largely fallen out of favour (19).

Selective IgA deficiency is more likely to coexist with CD compared to the general population (approximately 2% vs. 0.2% in non-celiac controls) (20). For this reason, it is generally recommended that a total IgA level should be checked along with IgA-based serologic celiac tests. While some individuals will have true selective IgA deficiency with undetectable levels of serum IgA, a larger number will have detectable but low levels of IgA (21). In those with detectable but low IgA levels, the accuracy of IgA-based tests was assumed to be acceptable. IgG AGA testing remained the standard diagnostic test for CD in individuals with selective IgA deficiency until recently when IgG anti-endomysial antibody (EMA), anti-tissue transglutaminase (anti-tTG), and anti-deamidated gliadin peptide (anti-DGP) assays were developed and proven to be superior to IgG AGA for this population (22).

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Detection of IgA and IgG anti-endomysial antibodies using an indirect immunofluorescence technique is another screening test with a sensitivity ranging from 87% to 93% and, in some reports, specificity exceeding 99% (19). The major problems of routine use of anti-endomysial antibodies are costs and the standardization of methods. Unlike anti-gliadin antibodies and, more recently, anti-tTG assays, which are based on the enzyme-linked immunosorbent assay (ELISA), the EMA assay is based on immunofluorescence. This requires either monkey esophagus or human umbilical cord tissue as a substrate plus microscopic examination of the sample. These add significantly to the costs and lead to concerns about inter-observer and inter-site variability (23).

With the identification of tTG as the target of CD autoantigen, the development of ELISA tests, which are easy to perform, became possible. In older children and adults, and when using assays utilizing human recombinant tTG, the IgA anti-tTG antibody screening test has 80-90% sensitivity and more than 95% specificity for diagnosing CD (24). In the developing immune system in younger age groups, these figures may vary (25). Results can be affected by the quality of commercially available kits (19). Several studies have investigated the variation in test results from assays by different manufacturers, demonstrating a significant range in test accuracy. Switching the assay to another one that is produced by a different manufacturer may be considered if a health-care provider is noting a poor test performance; when, for example there are major variations between serological testing and duodenal histopathology. Better-performing assays have a higher sensitivity than, and a specificity similar to, that of optimized

EMA testing, at a fraction of the cost and with a better reliability (26, 27).

1.1.3.2. Other serological diagnostic tests

Most recently, testing for antibodies against DGP (deamidated gliadin peptide) has become clinically available. This is based on the conversion of certain gluten peptides to deamidated peptides by the action of intestinal tTG. These peptides bind with high affinity to human leukocyte antigen DQ2 or DQ8 on celiac patients' antigen-presenting cells to potently stimulate the inflammatory T-cell response observed in the intestinal mucosa of patients with CD (22). Indeed, depending on the populations studied, IgA anti-DGP antibodies can be nearly as sensitive and specific as IgA anti-tTG antibodies (28, 29). However, recent studies have shown that IgA anti-tTG antibodies perform significantly better (30), and it currently is significantly less costly than IgA anti-DGP testing. On the other hand, whereas IgG anti-tTG testing has disappointing sensitivity (31), IgG anti-DGP and the composite IgA/IgG anti-DGP reach sensitivities above 80% and, importantly, specificities above 95% (28).

Because of cost and accuracy issues, on a population level, IgA anti-tTG testing with total IgA levels should be the first choice to be performed in patients investigated for CD. For these reasons, IgA anti-tTG testing is now the test of choice for diagnosis and monitoring of CD in most countries.

There seems to be little benefit to testing both anti-tTG and EMA simultaneously as the concordance rate of these tests is very high and individuals who test positive for either test should be referred for endoscopy (27).

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1.1.3.3. Histopathology

The current recommendation is to confirm the diagnosis of CD by small bowel biopsy specimens (18). The most widely accepted set of diagnostic criteria for histopathological diagnosis of CD is called the modified Marsh classification (32). This classification uses a combination of intraepithelial lymphocyte counts and description of the crypts and villi of the small intestine (Table 1). Although abnormal small bowel biopsy specimens are not specific for CD, in the appropriate clinical setting, abnormal biopsy specimens confirm the diagnosis. As the disease is patchy, the current guidelines suggest that at least 4-6 duodenal biopsies should be taken during EGD to prove the diagnosis (18). Marsh type 1 or higher is considered diagnostic for CD (32).

Table 1: The modified Marsh classification (32)

	Type 0	Type 1	Type 2	Type 3a	Type 3b	Type 3c
IEL*	<40	>40	>40	>40	>40	>40
Crypts	Normal	Normal	Hypertrophic	Hypertrophic	Hypertrophic	Hypertrophic
Villi	Normal	Normal	Normal	Mild atrophy	Marked atrophy	Absent

* Intraepithelial lymphocyes/100 enterocytes

1.2. Inflammatory bowel disease:

Inflammatory bowel disease (IBD) encompasses two related but distinct disorders of as yet unknown etiology: Crohn's disease (CrD) and ulcerative colitis

(UC). Crohn's disease is a chronic, idiopathic, transmural, patchy, inflammation of one or more segments of the digestive tract. Ulcerative colitis is a chronic, idiopathic, diffuse mucosal inflammation of the colon. Indeterminate colitis (IC) is reserved for cases of colitis in which findings are not sufficient to allow differentiation between CrD and UC (33).

1.2.1. Epidemiology

Inflammatory bowel diseases are ranked among the 5 most prevalent gastrointestinal diseases in the United States (34). The incidence of IBD appears to have risen over the last twenty years (35). However the incidence of CrD may now have plateaued and that of UC may be increasing (34). UC and CrD are diseases of young people with a peak incidence between the ages of 10 and 40 years. Twenty five percent of all cases usually present in children and young people (34). The only prospective national survey of IBD in children aged <16 years in the UK found an incidence of 5.2 per 100,000; 60% of those had CrD, 28% had UC and 12% had indeterminate colitis (36). The mean age at diagnosis was 11.9 years. Inflammatory bowel disease was slightly more common in boys and there was a slightly higher rate of UC in Asian children than in other ethnic groups (36). A systematic review of epidemiological studies of North American cohorts estimated the incidence of IBD at 3–4 per 100,000 individuals per year (37). A recent study from Canada showed that Ontario has one of the highest incidences of pediatric-onset IBD (11.4/100000) (38). Projected estimates suggest that up to 240,000 people are affected by IBD in the UK (39). An increase in IBD

incidence has been observed recently in Western and Southern Europe and in Asia (38).

1.2.2. Pathogenesis

The etiologies of both UC and CrD are unknown, but inflammatory bowel diseases are considered to be diseases of immune dysregulation, occurring in patients with the appropriate genetic predispositions. The consensus is that both diseases are probably a response to environmental triggers (infection, drugs, or other agents) in genetically susceptible individuals. The genetic component is stronger in CrD than in UC. Smoking increases the risk of CrD, but decreases the risk of UC through unknown mechanisms (40).

1.2.3. Diagnosis

In 2005, the IBD working group of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) developed a consensus protocol for investigating children with suspected IBD (33). The diagnosis of IBD is confirmed by clinical evaluation and a combination of biochemical, endoscopic, radiological, histological, and nuclear medicine investigations. The typical symptoms include abdominal pain, diarrhea with or without bleeding per rectum, and weight loss. The diagnosis of UC is made on clinical suspicion supported by appropriate macroscopic findings on colonoscopy, typical histological findings on biopsy, and negative stool examinations for infectious agents. For CrD, the diagnosis depends on demonstrating focal lesions with transmural inflammation

and the characteristic histopathological pattern (33).

1.2.4. Measuring disease activity

Both adult and pediatric investigators have recognized the need to optimize and standardize methodology for assessment of disease activity in clinical trials. For pediatric clinical trials, measures of disease activity were developed and validated for assessment of response to treatment and disease outcome.

1.2.4.1. Pediatric Crohn's Disease Activity Index (PCDAI)

The Pediatric Crohn's Disease Activity Index (PCDAI) is a multi-item instrument that consists of 4 general fields: history, physical examination, growth parameters and common laboratory tests (41). From this, a score is derived that ranges from 0-100. Cutoff values vary somewhat between different studies; however, a recent systematic appraisal of PCDAI concluded that a complete remission should be defined as having a PCDAI < 10 (42). A score of 10-30 reflects mild to moderate disease, and a score of >30 reflects moderate to severe disease (41). The detailed index items are found in appendix 1a.

1.2.4.2. The Pediatric Ulcerative Colitis Activity Index (PUCAI)

For UC, the Pediatric Ulcerative Colitis Activity Index (PUCAI) was recently developed and validated (43). It is based on 6 clinical items: abdominal pain, rectal bleeding, consistency of stools, frequency of stools, nocturnal stools, and general activity level. Values range from 0-85; a complete remission is

defined as PUCAI < 10 points (43) and a relapse in UC is defined as a PUCAI of >10. A score of 10-20 is considered as a mild disease, 20-35 is considered moderate, and a score of higher than 35 is considered a severe disease (43). The detailed items are found in appendix 1b.

1.3. Relationship between celiac disease and inflammatory bowel disease
1.3.1. Anti-tissue transglutaminase antibodies and inflammatory bowel disease activity

There is some evidence that immune-mediated diseases cluster together (44, 45), and that such clustering might also occur between IBD and CD. A genome scan study showed non-random clustering of susceptibility loci of autoimmune diseases supporting the clinical impression that these autoimmune diseases might cluster together in individual patients (46).

The serologic diagnosis of CD has been based on the detection of certain antibodies, including anti-tTG IgA antibodies. However, these antibodies also are present in other immune disorders. Di Tola et al examined the levels of anti-tTG IgA antibodies in a cohort of patients with IBD. They recruited a total of consecutive 78 adult patients with IBD (49 CrD and 29 UC). Activity indices for CrD and UC were calculated. A group of 45 untreated celiac disease patients was also recruited along with 85 patients with other autoimmune problems including insulin-dependent diabetes (IDDM) and multiple sclerosis (MS) and 58 healthy volunteers as control groups (47).

Anti-tTG IgA antibodies and Anti-endomysial antibodies (EMA) were

measured in all study participants. In the celiac group patients, 44 (98%) had anti-tTG positive IgA antibodies; half of them were strongly positive and the other half was weakly positive. In the IBD group, 52 patients (66%; 32 patients with CrD and 20 patients with UC) had low-positive response to anti-tTG IgA antibodies (47). The authors did not provide clear definitions of "strongly positive" versus "weakly positive". In patients with other autoimmune disorders, only one patient with multiple sclerosis tested low positive. Healthy controls were all negative for anti-tTG IgA antibodies. While there was no difference in the mean levels of anti-tTG IgA antibodies between patients with CrD compared to those with UC, the anti-tTG IgA antibody levels were significantly higher in the celiac group. Interestingly, while all patients with celiac disease had positive EMA, none of the other patients who had IBD or other autoimmune disorders had positive EMA. No healthy controls had positive EMA. There were significant correlations between anti-tTG IgA antibody levels and IBD activity indices (r=0.77, p<0.001 for CrD and r=0.69, p<0.001 for UC). No patients had esophagogastroduodenoscopy (EGD) with duodenal biopsies done to confirm the diagnosis of celiac disease (47).

1.3.2. Clinical evidence

Several reports have shown an association between CD and IBD, especially UC (48-57). Siblings to patients with celiac disease seemed to have 15-fold increase in the risk for developing UC but did not appear to be in a higher risk for developing CrD (54). Shah et al found a prevalence of 697/100,000 of IBD

among first degree relatives of patients with CD compared to the expected prevalence of 150/100,000 (55). Cottone et al reported a 10- time increase in the familial incidence of IBD among first-degree relatives of patients with CD compared to those without (56).

Tursi et al reported a high prevalence of CD in adult patients with IBD (52). They examined the prevalence of celiac disease in 27 patients newly diagnosed with Crohn's disease (mean age 32.3 years, range 16-69 years, 13 men). The investigators performed screening tests in the form of serum anti-gliadin, anti-endomysial and anti-tTG antibodies and sorbitol H2 breath test on these patients (52). Patients with positive tests had EGD performed with 6 small bowel biopsies taken from the second part of the duodenum. Eleven patients (40%) had EGD based on one or more positive screening tests.   Out of these 11 patients, nine had abnormal histopathological findings in their duodenal biopsies, five of whom had changes consistent of CD. Thus, five out of 27 patients with Crohn's disease (18.5%) had histopathological diagnosis of celiac disease (Marsh III). These five patients had positive anti-tTG IgA antibodies and 4 of them had positive EMA. The rest of 27 patients with Crohn's disease had negative anti-tTG IgA antibodies. The authors did not provide a reasonable justification of this high prevalence of celiac disease in their cohort. Instead, they focused on discussing general principles of common immunology background for both diseases (52). It is not clear in this study what the time interval between initial diagnosis of Crohn's disease and the diagnosis of celiac disease was i.e. when they performed EGD (52).

Yang et al reviewed their database to find patients with biopsy-proven

celiac disease and then examined the prevalence of IBD in these patients (53).

Among 445 patients, 10 (2.2%) met the diagnostic criteria for IBD (5 had CrD

and 5 had UC). To determine if the prevalence of IBD was increased in their

cohort of patients with CD compared to general population, they calculated the

age and sex-adjusted prevalence rate ratio of both CrD and UC using population-

based data for the United States. They reported an age and sex-adjusted

prevalence rate ratio of 3.56 (95% CI 1.48-8.56) for UC and 8.49 (95% CI 3.53-

20.42) for CrD (53) i.e. patients with CD have a 3.6 times increase in the

likelihood of UC and 8.5 times increase in CrD.

In a large study from the UK, Leeds et al estimated the prevalence of celiac

disease in 354 adult patients with known IBD (209 females, median age of 45

years). They also examined the prevalence of IBD among 305 adult patients with

known CD (222 females, median age of 52 years). A control group of 601 healthy

adults (391 females, median age 47 years) was recruited (58).

Celiac disease status in patients with IBD was examined through sending

blood samples for IgA and IgG AGA antibodies, IgA EMA and IgA anti-tTG

antibodies. Out of the 354 patients with IBD, 45 (13%) patients tested positive to

one or more of all serological screening tests for celiac disease. Only 3 patients

(0.9 %) had villous atrophy in their duodenal biopsies (consistent with the

diagnosis of CD). All three of them had anti-tTG positive IgA anti-bodies and

only two of them had EMA positive antibodies. One patient had CrD and the

other 2 had UC. One patient with positive EMA and anti-tTG antibodies declined

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having EGD.

The prevalence of celiac disease among the healthy control group was 0.8% (5 patients out of 601 patients). There was no significant difference between patients with IBD and healthy controls regarding the prevalence of CD (odds ratio (OR)) 1.02, 95% CI, 0.24-4.29, p=1.0). On the other hand, the prevalence of IBD in celiac disease patients, based on ileocolonoscopy with biopsies, was 3.3% compared to 0.3% in the control group (OR 9.98, 95% CI, 2.8-45.9, p<0.001) (58). Despite small number of patients with IBD who were found to have CD, the authors used multivariate regression analysis to identify factors that were likely to predict development of CD in IBD. Only positive EMA and anti-tTG IgA antibodies were found to be significant (p<0.001). The study concluded that celiac disease patient had a 10-fold increased risk to develop IBD while patients with IBD were not at any increased risk to have celiac disease (58). Using these antibodies in the logistic regression model was a major flaw in that study as these antibodies are predictive of celiac disease in any individual. The authors did not mention the details of initial endoscopic or histopathological findings of their cohort or the levels of different antibody screening tests for celiac disease. IBD activity indices were not examined in their regression model (58).

In an uncontrolled retrospective analysis, Mantzaris et al reported a prevalence of CD in 281 adult patients (mean age of 25 years) with Crohn's disease to be 0.4% (one patient) (59). Interestingly, 46 patients had IgG AGA positive antibodies and the majority of them (36 patients) had duodenal histopathological changes consistent with Crohn's disease rather than CD. It was

not clear what histopathological features of CD or Crohn's disease in these patients were documented. Data for anti-tTG IgA antibodies were available for 160 patients and 4 (2.5%) of them were positive (59). The same group reported a prevalence of celiac disease in 358 consecutive patients with ulcerative colitis to be 1.4% (5 patients). No further details regarding histopathological duodenal features, serological screening tests or IBD activity were reported. It is not clear if all patients at the time of screening for celiac disease were on gluten containing diet or not. This may well have affected the results of this study and made It difficult to use it in any substantive way (59).

In a recent multi-centre Italian study, Casella et al enrolled 1711 consecutive adult patients with IBD; 860 had Crohn's disease, 791 had UC and the rest had indeterminate colitis (60). Patients were screened for celiac disease through measuring EMA and anti-tTG IgA antibodies. Patients had EGD performed with duodenal biopsies if the serological screening tests were positive or they were IgA deficient. Nine patients (0.5%) were diagnosed with celiac disease based on positive serological and histopathological findings (Marsh II-III). Six of these patients had UC and 3 had Crohn's disease. Out of these 9 patients, 8 were positive to both EMA and anti-tTG IgA antibodies while one patient was only positive to EMA (60). None of the IgA deficient patients was found to have celiac disease in EGD. This study showed a low prevalence of celiac disease in patients with IBD (60). However, it is not clear if all patients at the time of screening for CD were on gluten containing diet or not. It is not uncommon for patients with IBD to avoid gluten containing food thinking that

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this may improve their IBD symptoms. Again, although the investigator

mentioned in their methods that the activity indices of the IBD were recorded,

these data were not provided (60).

Bizzaro et al screened 170 adult patients (100 with UC and 70 with

Crohn's disease) with IBD for CD using IgA and IgG anti-tTG antibodies (61).

Those who were positive had EMA measured. If either was positive, they had

EGD performed with duodenal biopsies. They also screened 120 healthy

individuals a control group. Only one patient with UC and one healthy control had

positive anti-tTG IgA antibodies. Both of them had celiac disease confirmed by

EGD and duodenal biopsies. No disease activity or antibody levels were

described. No histopathological description of duodenal biopsies was provided.

No Diet description or gluten intake was provided (61).

In an adult study form the UK, Dahele et al, examined the presence of anti-

tTG IgA antibodies in 116 patients with untreated celiac disease, 82 patients with

treated celiac disease, 65 patients with suspected celiac disease (normal

individuals on endoscopy), 163 patients with IBD (82 with UC and 81 with CrD)

and 29 health volunteers (62). Anti-gliadin antibodies and EMA were also

measured. IgA levels were also measured for all patients and 2 patients with IgA

deficiency were excluded. Four patients with IBD (2.5%); 2 with CrD and 2 with

UC had positive anti-tTG IgA antibodies. No IBD disease activity was measured

(62).

In summary, the data regarding any correlation between IBD disease

activity and anti-tTG IgA antibodies levels are scarce but do show some evidence

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of correlation between IBD disease activity and anti-tTG IgA antibodies levels.

Table 2 summarizes the findings. The majority of the published data did not show

any increase in the prevalence of celiac disease in patients with IBD. Overall, all

the studies that have been conducted so far examining this hypothesis have only

involved adult patients with no pediatric data.

Table 2: Summary of literature (CD and IBD)

Paper	Design	N	Outcome
Tursi (52)	Single center (Italy) prospective case series	27 adults of newly diagnosed CrD	18% had CD based on histopathology
Yang (53)	Single center (USA) retrospective cohort	445 adults with CD	10 patients with IBD (5 UC; adjusted prevalence rate ratio 3.56 and 5 CrD adjusted prevalence rate ratio 8.49%)
Leeds (58)	Single (UK) prospective cohort	354 adults with IBD, 305 CD and 601 controls	12 in IBD group had positive celiac screen, 3 had histopathological features of CD and 5 in the control group had CD
Mantzaris (59)	Single center (Greece) retrospective series	281 CrD and 358 UC.	In CrD patients, 2.5 % had positive celiac screen, 0.4% had histo-pathological diagnosis. In UC patients, 1.4% had CD but it was not clear how the diagnosis was made
Casella (60)	Multiple centers (Italy) prospective series	Adults 1171 adults with IBD	9 patients (0.5%) had CD based on serology and histopathology.

1.3.3. Molecular evidence

At the molecular level, both CD and IBD affect the intestinal barrier, which functions to regulate transport and the host's defensive mechanisms by separating the foreign environment of the intestinal luminal contents from the rest of the body. The anatomical barrier includes physical structures of the mucosa, while the immunological barrier focuses on defense against foreign antigens and combines both innate and adaptive immunity. Breaching either of these barriers may lead to disturbed homeostasis and eventually to a disease state (63).

Increased intestinal permeability in both disorders suggests that alterations in tight junction structure and physiology are part of a common pathogenesis, which could be due to shared genetic variants (63). Indeed, genes that might influence intestinal permeability were associated with both celiac disease and IBD (63). Although genetic barrier defects are likely part of a common etiology, it is still important to realize that the observed increase in permeability in both disorders is largely determined by the consequential intestinal pathology. The inflammatory processes in IBD and celiac disease seem to be comparable to some extent, even though they are instigated by different antigens: a small amount of antigen starts an acute mucosal inflammation which leads to increased permeability, influx of larger quantities of antigen and extensive mucosal damage.

Predisposition to the enhancement of any component of this inflammatory cycle could predispose a person to either disease. Impaired pattern recognition receptors that allow bacteria to cross the epithelial boundary without detection can lead to enhanced inflammation and increased permeability in both diseases. In

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IBD, this will lead to the translocation of more bacteria, while in celiac disease it allows the influx of more gluten, and hence more inflammation. This also applies in both diseases to the induction of pro-inflammatory cytokines, resulting in increased permeability and subsequent increased inflammation (63). There is some evidence for a common congenital predisposition for such an enhanced inflammatory reaction since IL18RAP polymorphisms are associated with both diseases (63). Further research devoted to the genetic predisposition for IBD and celiac disease may yield more shared inflammatory genes. Overall, it has been concluded that the epidemiological overlap between IBD and celiac disease is probably caused by common genetic predispositions for both an impaired epithelial barrier and enhanced immunological sensitivity to luminal antigens (63). Recent hypotheses have suggested that potential genetic factors predisposing patients for both conditions may lead to a possible association between both conditions (64, 65). Moreover, genetic variation in the chromosome 4q27 region (associated with celiac disease and some other autoimmune disorders) predisposes to UC, suggesting a common genetic background for both diseases. Nonetheless, some other well-established risk factors for IBD were not demonstrated to be candidate genes for development of celiac disease (66).

1.4. Summary and proposal

Overall, although theoretically speaking, there seems to be a link between IBD and celiac disease, epidemiological and clinical data are conflicting. As well, all available data are based on adult literature. Currently, it is not clear whether

serological blood tests for screening for CD are affected by inflammatory bowel disease activity in patients with IBD or not. Only one study has investigated that and a positive correlation between IBD activity and CD serology status was found (47). This study was an adult study and, to date, no pediatric data are available.

While the prevalence of CD in patients with IBD was 18% in one study (52), other studies have failed to show a similar increase in the prevalence of CD in patients with IBD (58, 59). On the other hand, it seems that IBD is more prevalent in patients and families with CD (50, 53, 56). Again, all the available data are adult data and pediatric evidence is currently lacking.

1.4.1. Rationale:

Most patients with celiac disease and IBD may share common symptoms and signs (e.g. diarrhea, abdominal pain and iron deficiency anemia). Missing one disease in a patient with 2 diseases may lead to serious complications (e.g. severe malnutrition and growth faltering) and long standing untreated problems (48,49,51). It may lead to an assumption of a poor control of one disease and possibly inappropriate therapy and the use of unnecessary medications with potentially serious side effects. Hence, it is important to determine:

if changes in the activity of inflammatory bowel disease affect anti-tTG Ig A antibody levels;

if there is a significant overlap/association between celiac disease and inflammatory bowel disease; and,

if there is a significant association between IBD and CD, and if so, how

common is this association.

These associations have not been explored yet in the pediatric population. The aim of my research was to (a) examine whether anti-tTG IgA antibody titers in children vary with changes of IBD activity or not, and, (b) examine a possible increase in the prevalence of celiac disease in children with IBD. This should enhance our understanding of the pathophysiology of IBD and affect IBD management (i.e. looking for celiac disease in patients with IBD who are difficult to control).

1.4.2. Objectives

To determine if serological markers of celiac disease vary with changes in the IBD disease activity in children.

1.4.3. Hypothesis

The hypothesis being tested is that a change in the disease activity index would correlate with a change in anti-tTG IgA antibody titers. This can be expressed as:

Null Hypothesis (H_o): Changes in anti-tTG IgA antibody titers do NOT correlate with changes in inflammatory bowel disease activity indices.

Alternative Hypothesis (H_A): Changes in anti-tTG IgA antibody titers correlate with changes in inflammatory bowel disease activity indices.

As a secondary outcome, the same sample is used to examine the prevalence of

celiac disease in children with IBD compared to those without IBD.

The hypothesis being tested is:

Null Hypothesis (H_o): Prevalence $_{IBD}$ = Prevalence $_{Non-IBD}$

Alternative Hypothesis (H_A): Prevalence $_{IBD}$ is not equal to Prevalence $_{Non-IBD}$

Chapter 3   Patients and methods

2.1. Study design

The study was designed as a single-centre, hospital-based, prospective cohort, pediatric study. Children (patients and controls) were recruited prospectively and consecutively from IBD and general gastroenterology clinics at the Stollery Children's Hospital, Edmonton, Alberta.

2.2. Recruitment

2.2.1. Patients

Patients were children, 2-18 years of age, coming to the IBD clinics or ER at the Stollery Children's Hospital with established diagnosis of IBD based on clinical, radiological, and endoscopic evidence. Patients were recruited regardless of the disease activity. They were recruited consecutively between September 2007 and January 2010. Inclusion/exclusion criteria are listed in table 3.

2.2.2. Controls

Age-matched controls (2-18 years) were recruited at a 1:1 ratio with cases. They were recruited from the general gastrointestinal clinics at the Stollery Children's Hospital. The control group consisted of children with functional gastrointestinal problems e.g. irritable bowel syndrome, with no evidence of celiac disease or IBD. Inclusion/exclusion criteria are listed in table 3.

Table 3: Patients and controls eligibility criteria

Inclusion Criteria	Exclusion Criteria
Children 2-18 years	Children < 2 years and adults >18 years
For patients: Diagnosis with IBD (CrD and UC)	Other causes of colitis/indeterminate colitis
For controls: Do not have IBD.	On Gluten-free diet
On gluten-containing diet	
	Patients known with IgA deficiency, thyroid problems, Down syndrome, Turner's syndrome, William's syndrome and first degree relatives for patients with celiac disease

2.3. Data Collection

2.3.1. Data collection for patients

After reading the study information sheet (Appendix 2) and having the study explained to patients and their parents, patients were asked on their clinic/ER visits to provide an informed consent to participate. Data were collected from patients and recorded on a patients' data collection sheet (Appendix 3)

Blood samples for anti-tissue transglutaminase IgA antibodies and IgA levels were collected in addition to the other routine blood work (such as a complete blood count (CBC, including haemoglobin level and white blood cell and platelet counts) erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum albumin) at least twice at 2 different clinic visits. Patients with positive anti-tTG IgA antibodies had EGD and duodenal biopsies with

histopathology performed. All patients had anti-tTG IgA antibodies measured in 2 different occasions at least 3 months apart.

Screening for celiac disease was performed through measuring serum IgA anti-tTG antibodies at 2 different time points using an ELISA method based on recombinant human tTG (Celikey; Pharmacia Diagnostics, Freiburg, Germany). The coefficient of variation (CV) of the assay is 7%. Dietary history was carefully assessed to confirm the presence of gluten in patients' diet.

2.3.2. Data collection for controls

Data were collected from controls (Appendix 4: controls data collection sheet).
Inflammatory bowel disease was excluded in control children through history, clinical examination, and investigations which included CBC, inflammatory markers (CRP and ESR), serum albumin levels, and esophagogastroduodenoscopy (EGD) with or without colonoscopy, if clinically indicated.

Controls were collected consecutively and prospectively from the general gastroenterology clinics during the study period and were children diagnosed with functional gastrointestinal problems, mainly irritable bowel syndrome and functional dyspepsia. Dietary history was carefully assessed to confirm the presence of gluten in control's diet.

A blood sample for anti-tissue transglutaminase (Anti-tTG) IgA antibodies with IgA levels (using the same laboratory method for patients) was added to

other routine blood investigations that were collected. Controls with positive anti-tTG IgA antibodies had EGD with duodenal biopsies and histopathology performed.

2.4. Assessment of IBD activity

Data (form 1: "data collection sheet" in Appendix 4) collected from patients were used to calculate inflammatory bowel disease activity indices; pediatric ulcerative colitis activity index (PUCAI) (43) and pediatric Crohn's disease activity index (PCDAI) (41) (Appendix1) were calculated. The calculation was performed by a staff gastroenterologist during two different clinic visits for each patient at least 3 months apart.

Information regarding clinical symptoms and signs of active disease (diarrhea, abdominal pain, bleeding/rectum, and weight loss), and laboratory measures (e.g. hemoglobin, inflammatory markers and serum albumin) were collected. Other information collected included disease distribution, duration of illness, and current medications used.

2.5. Sample size determination & statistical analysis

It was determined that a total sample of 328 children (164 children in each arm) would provide over 90% power to detect a change of 0.5 in the mean anti-tTG IgA antibody levels using a two-sample unpaired Student's t-test.

Assumptions:

I determined that a sample size of 137 children in each arm would give a

power of 94 % provided that the mean of anti-tTG IgA antibody levels of patients is 0.5 (SD=1), and the mean of controls is 1 (SD=1), and α=0.05. A sample size 85 children in each arm would give a power of 90 % provided that the mean of anti-tTG IgA antibody levels of the first group is 0.5 (SD=1), the mean of the second group is 1 (SD=1), and α=0.05.

The change of 0.5 was selected to detect any minor changes in serology levels. The reported coefficient of variation of the serological test used was 7%. I determined that a sample size of 163 patients would provide over 90 % power to detect R^2 value of > 7 % (i.e. 7% of the variability in serology is explained by IBD activity) using multiple linear regression. Age as variable was examined in the regression model as a possible confounder.

The sample size calculation was mainly performed for aim number 1and not for aim number 2 of the study. A significance level of 0.05 was used in all calculations. Calculations and data analysis were performed using STATA 9.1 ™ (StataCorp. 2005. *Stata Statistical Software: Release 9*. College Station, TX: StataCorp LP). Univariate summaries (means, ranges, and SD) were calculated for each group (patients and controls) for continuous variables (age), while frequencies were calculated for categorical variables, along with 95% confidence intervals (CIs) for the means and proportions. Variables were examined for normal distribution. Student's t-test was used to compare means.

Study aim number 1 was examined by retaining both serology results and IBD activity results in their continuous state. The change in anti-tTG IgA antibody levels between the 2 time points (response) was regressed against the

change in IBD activity (predictor) at the same 2 time points using multiple linear regression with "age" inserted as a variable to control for its potential confounding effect.

The means of the anti-tTG IgA antibodies obtained at the 2 different time points, (response) were regressed against the means of the activity indices measured at the two same time points, (predictor), adding the age variable to the regression model as a possible confounder.

For study aim number 2, the chi-square test was used to compare the prevalence of celiac disease among patients to that among the controls.

2. 6. Data management

Data were collected using standardized data collection forms as described previously (Appendices 3&4). Data were entered into a spreadsheet (Excel, Windows 2007), on a weekly basis without intermediate coding.

2.7. Ethics

The study protocol was approved by the University of Alberta Health Research Ethics Board (Appendix). A copy of the Parent's Information sheet is provided in the appendix.

Chapter 3   Results

3.1. Descriptive analysis

One hundred and sixty four children with IBD (85 CD, 79 UC) were recruited from the Pediatric IBD clinic at the Stollery Children's Hospital, Edmonton, AB, between September 2007 and January 2010. Three patients declined informed consent for the study. The mean age for patients was 14.1 (SD 2.95), range 3.6 to 17.3 years. Mean duration of illness was 3.12 years (SD 2.84), range 0.2-14.9 years). The interval between the first and the second time of assessment ranged between 0.1- 2.1 years. Demographics of patients and controls, and study variables are summarized in table 4.

Table 4: Summary of variables examined

Variable	N	Mean	SD	Range	Unit
Age (patients)	164	14.10	2.95	3.6-17.3	year
Age (control)	164	12.37	3.71	4.1-17.1	year
Duration of IBD	164	3.12	2.84	0-14.9	year
Anti-tTG antibodies (patients) Time 1	164	1.18	0.78	0.3-7.2	U/L
Anti-tTG antibodies (Controls)	164	3.96	34.20	0.5-439	U/L
PUCAI 1	79	16.40	19.30	0-65	points
PCDAI 1	85	21.71	18.32	0-60	points
Anti-tTG antibodies (patients) Time 2	164	1.62	1.20	0.1-13	U/L
PUCAI 2	79	4.82	10.04	0-60	points
PCDAI 2	85	17.11	15.01	0-82.5	points

3.2. Correlation between the changes in disease activity indices and anti-tTG IgA antibody levels

The difference between the PCDAI obtained at first and second time points was calculated. Similarly, the difference between in anti-tTG IgA antibody levels collected at first and second time points was calculated. The correlation between the differences was not significant (r = 0.004) (Figure 1).

The difference between the PUCAI obtained at first and second time points was calculated as was the difference between changes in anti-tTG IgA antibody levels. There was no significant correlation between the two variables (r = 0.02) (Figure 2).

3.3. Regression analysis for the changes in disease activity indices and anti-tTG IgA antibody levels

Linear regression analysis was performed between the difference in activity indices at the two time points and difference in anti-tTG IgA antibody levels at the same time point. Age was examined as a possible confounder and proven not to be (p > 0.20). As shown in table 5, there was no significant effect of changes in disease activity indices on changes of anti-tTG IgA antibody levels at the two points of time.

3.4. Regression analysis for the absolute values of activity indices and anti-tTG antibody levels.

Linear regression analysis was performed to examine any possible

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relationship between absolute values of activity indices and absolute values of anti-tTG antibody levels. There was no significant correlation between the absolute values of PCDAI and anti-tTG IgA antibody levels at the first time point (ß coefficient = 0.80, p = 0.7) or at the second time point ((ß coefficient = 1.63, p = 0.3). Similarly, there was no significant correlation between absolute values of PUCAI, age and anti-tTG IgA antibody levels at the first time point ((ß coefficient=0.96, P=0.3 or at the second time point ((ß coefficient = 1.34, p = 0.1).

3.5. Difference between patients and controls in anti-tTG IgA antibody levels

Using the unpaired Student's t test, there was no statistically significant difference between patients and controls in the mean anti-tTG IgA antibody levels at 1st time point (p = 0.3) or at the 2nd time point (p = 0.4).

3.6. Prevalence of celiac disease in children with IBD

Only one patient with IBD (UC) had positive anti-tTG antibodies at 7.2 units/ml then 13 units/ml and his celiac disease was confirmed endoscopically (Marsh III b). The duodenal biopsies at the initial diagnosis of IBD were not consistent with celiac disease. In the control group, one patient had positive anti-tTG IgA antibodies (value: 439 Units/ml) and the diagnosis of celiac disease was confirmed via endoscopy and small bowel biopsies (Marsh III c). Consequently, the prevalence of celiac disease in the patient group and control group was similar (1/164 (0.6%)). There was also no statistically significant difference between celiac disease prevalence in patients and controls (chi-square test p > 0.05).

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Figure 1: No correlation between changes in PCDAI and changes in anti-tTG antibodies
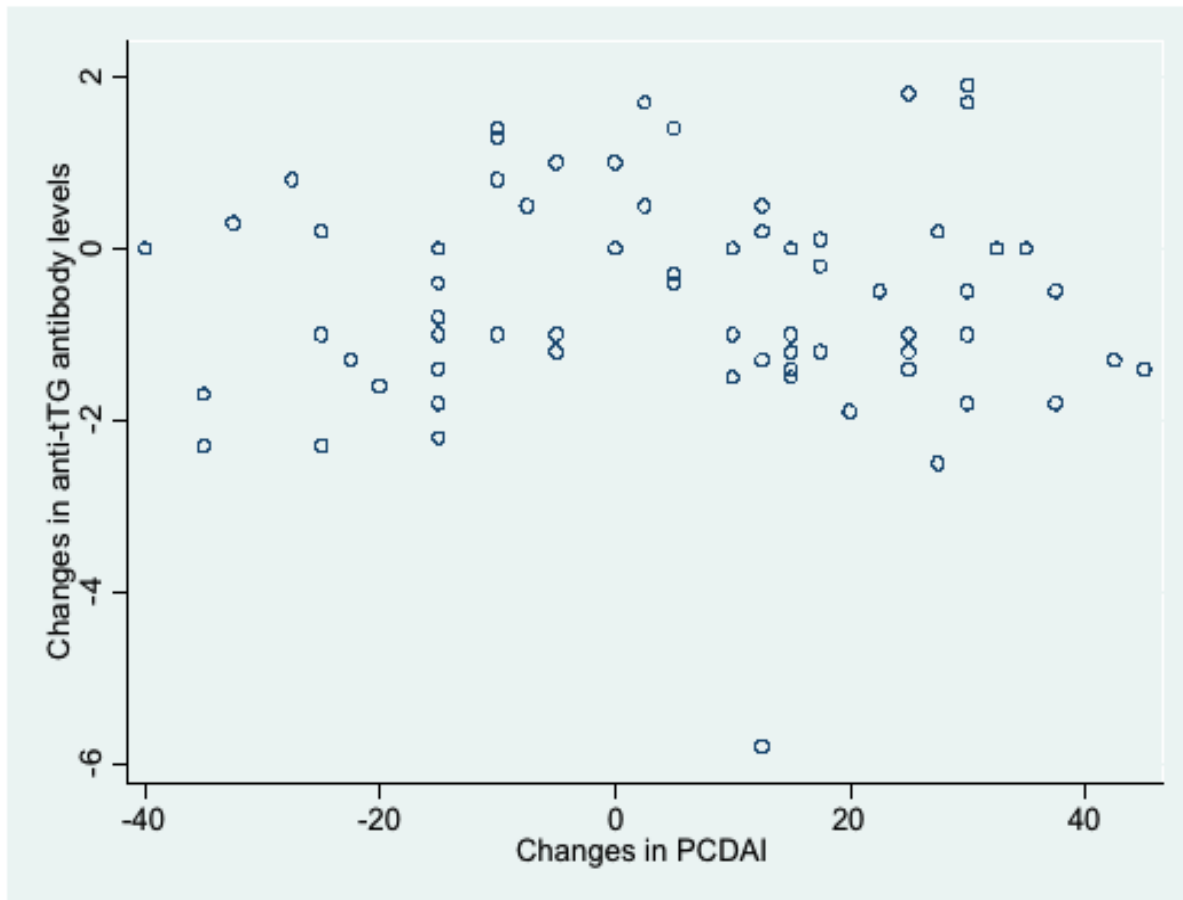
* Note the one outlier indicates a patient who had a high tTG level

Note that negative values (negative change) indicate that the PCDAI at second time point was higher than first PCDAI i.e. worsening of CrD

Figure 2: No correlation between changes in PUCAI and changes in anti-tTG antibodies
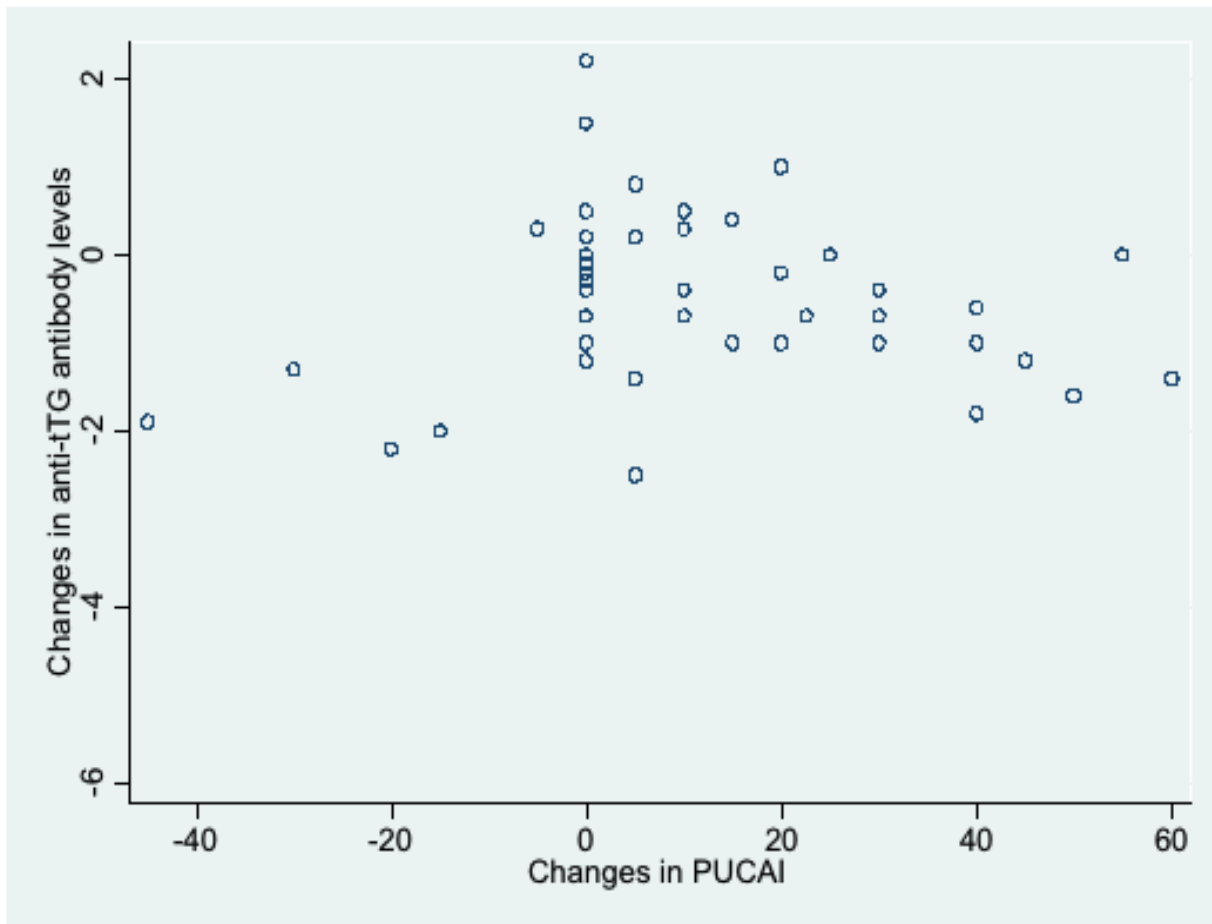
Note that negative values (negative change) indicate that the PUCAI at second time point was higher than first PUCAI i.e. worsening of UC

Table 5: Multivariate linear regression analysis showing no relationship between changes in IBD activity indices and changes in anti-tTG IgA antibodies in children with IBD

	Difference in anti-tTG IgA antibodies		
	ß Coefficient	95% CI	P value
Difference in PUCAI	-0.91	-7.3, 5.49	0.7
Age (for UC patients)	0.7	-0.94, 2.35	0.4
Difference in PCDAI	-0.34	-4.25, 3.57	0.9
Age (for Crohn's patients)	1.11	-0.81, 3.03	0.3

Chapter 4 Discussion

The number of children being diagnosed with celiac disease (CD) is increasing; this may be due to greater recognition of the more atypical presentations, improved serologic tests, and the screening of asymptomatic groups at increased risk, but may also be due to an overall increased incidence of CD (25). In a prevalence study, Fasano et al estimated the overall prevalence of CD in the United States in not-at- risk groups to be 1: 133 (67).

The classical presentation tends to occur in younger children, while atypical presentations occur at an older age (25). Although serologic testing has become more reliable, there still remain significant problems around testing, particularly in those <18 months of age. All children should undergo a duodenal biopsy on a gluten containing diet in order to diagnose CD before recommending a gluten-free diet (25).

On the other hand, inflammatory bowel diseases (IBD), mainly ulcerative colitis and Crohn's disease, are chronic, heterogenic, lifelong illnesses with onset at a young age and a great potential for disability. The incidence of inflammatory bowel disease in the pediatric population is increasing (34, 35). Approximately, 20 % of IBD present before the age of 18 years old (35). The typical symptoms include abdominal pain, diarrhea with or without bleeding per rectum and weight loss (33).

Inflammatory bowel disease and celiac disease are both chronic inflammatory diseases of the intestinal tract and their pathogenesis is influenced by environmental as well as immunological and genetic factors (68). In both

diseases, an antigen activates several inflammatory pathways, which cause

extensive damage to the intestinal mucosa and lead to increased permeability of

the intestinal epithelium (68). The inflammatory processes in IBD and celiac

disease seem to be comparable to some extent, even though they are instigated by

different antigens: a small amount of antigen starts an acute mucosal

inflammation which leads to increased permeability, influx of larger quantities of

antigen and extensive mucosal damage. Predisposition to the enhancement of any

component of this inflammatory cycle could predispose a person to either disease

(68). There is a significant overlap between symptoms of CD and those of

inflammatory bowel disease. Consequently, it is logical to look for CD in patients

with IBD. Although there have been several adult reports that explored the link

between CD and IBD, no pediatric data are available.

In my study, one hundred and sixty four children with IBD (85 with

Crohn's disease and 79 with ulcerative colitis) were prospectively recruited. The

disease activity was assessed at two different time points using validated pediatric

tools; pediatric Crohn's disease activity index and pediatric ulcerative colitis

activity index. The serum levels of anti-tTG IgA antibodies were measured at

these two time points.

4.1. Correlation between changes in disease activity and changes in anti-tTG IgA

antibodies:

A possible correlation between any changes of disease activity indices and

changes in serum levels of Anti-tTG IgA antibodies was examined. No correlation

was found. Available data examining the same objective are scarce. However, my results are different from the results of the study by Di Tola et al (47) who recruited 78 patients with IBD and found significant correlation between disease activity indices and anti-tTG IgA antibody levels. The difference in results may be explained by the fact that Di Tola et al used different activity indices. Another major difference is all patients in the Di Tola et al study were adults (47). The quality of the kits that were used to measure anti-tTG IgA antibodies cannot be assessed. Of note is that all patients with IBD in that study were found to be EMA negative including those who were anti-tTG IgA antibody positive (47). Di Tola et al divided positive anti-tTG antibody results into "weak positive" and "strong positive". It was not clear what was the value of demarcation did they use. There is no evidence to suggest that timing of measuring of anti-tTG antibody levels would have a significant effect of the values of the titer levels.

4.2. Celiac disease prevalence in children with IBD

I also looked for any possible increase in celiac disease prevalence among 164 children with IBD. A control group of 164 children with functional gastroenterology problems was recruited. One child in each group was proven to have celiac disease. These two children had positive anti-tTG IgA antibodies and the diagnosis of celiac disease was confirmed via EGD and duodenal biopsies.

My results confirmed no increase in the prevalence of celiac disease in children with IBD. Over the last few years, a number of case reports and case series have suggested a possible association between celiac disease and IBD (48-

51). However, the majority of the studies that examined any possible association between IBD and celiac disease agreed with my results (53, 58-60). A recent uncontrolled Italian study concluded a high prevalence of celiac disease (18%) in adults patients newly diagnosed with Crohn's disease (52). The authors failed to provide convincing explanations for their findings. It was not clear in that study what was the time interval between initial diagnosis of Crohn's disease and the diagnosis of celiac disease (52). The pathological findings of duodenal biopsies from initial endoscopy were not mentioned.

The present study has several strong points. This is the first pediatric study that looked at the correlation between IBD disease activity indices and anti-tTG IgA antibodies. It is also the first pediatric study that examined the prevalence of celiac disease in children with IBD. The prospective design adds to the strength of the study. Data collection was thorough and meticulous. Although the study results confirmed the null hypothesis (a negative study), the results are important and add to the current knowledge.

4.3. Limitations:

The first limitation of the present work is the calculated sample size for the second aim of the study. The sample size may have been underestimated and 164 patients in each arm may not have been enough to a avoid type 2 statistical error. However, it is important to stress that this aim was not the main aim of the study and so the sample size calculation mainly addressed aim 1. Nonetheless, examining study aim 2 was a useful secondary exploration. To examine this

hypothesis with 80% power, a sample size of 2515 patients will be needed in each arm assuming that CD prevalence in IBD group is 2% versus 1% in the control group.

The second limitation is the control group recruited was a group of children with functional gastrointestinal disorders e.g. irritable bowel syndrome (IBS). Although it is better to recruit a control group with no gastrointestinal symptoms, there is conflicting evidence suggesting that celiac disease may or may not be more prevalent in patients with functional gastrointestinal problems. The prevalence of functional gastrointestinal disorders is much higher compared to the prevalence of celiac disease (69). In a pediatric study from the UK, IBS was the commonest cause of recurrent abdominal pain in children, affecting about one third of these children (69). While some studies have suggested that the prevalence of celiac disease is higher in patients with IBS compared to general population (70), other studies failed to confirm this finding (71,72).

In a case control adult study from the UK, Sanders et al reported the presence of celiac disease in 14 patients out of 300 patients with IBS based on Rome II criteria (70). They only found 2 patients with celiac disease in their healthy control group. Nonetheless selection bias was a major concern in their study (70).

On the other hand, another adult case-control study from the United States had a different conclusion (71). Locke at al recruited 84 patients with dyspepsia and/or IBS and 78 asymptomatic healthy controls to address the same question. They measured serum anti-tTG IgA and anti-endomysial antibodies for all

patients and healthy controls (71). Two patients in the IBS group and two patients in the asymptomatic volunteers had positive anti-tTG antibodies. Anti-endomysial antibodies were negative in all subjects. This result suggested that celiac disease was not more prevalent in patients with IBS and so, celiac disease did not explain the presence of patients' IBS or dyspepsia (71).   Both studies used serum markers as a tool for diagnosing celiac disease (70-71).

In an uncontrolled adult study from Norway, El-Salhy et al, performed anti-tTG antibodies and EGD on 968 patients with IBS based on Rome III criteria (72). Only 4 patients were positive for ant-tTG IgA antibodies (0.04%). The diagnosis of celiac disease was confirmed with EGD and duodenal biopsies in 4 patients. One patient had Marsh III small bowel pathological changes while the remaining 3 patients had Marsh I small bowel changes (72). The study concluded that prevalence of celiac disease in their cohort was less than the reported figures for the prevalence of celiac disease in the general population. A weak point was the study design as it was an uncontrolled study. On the other hand, a strong point in that study was the utilization of the gold standard for diagnosing celiac disease; EGD and duodenal biopsies (72).

Chapter 5   Conclusions

Using validated pediatric inflammatory bowel disease activity measuring tools, the study found no correlation between changes in inflammatory bowel disease activity and changes in anti-tTG IgA antibody titer levels at two different time points. Consequently, anti-tTG IgA antibody titer levels can't be used to monitor inflammatory bowel disease activity in children.

Despite the theoretical evidence linking celiac disease and inflammatory bowel disease, the prevalence of celiac disease in children with inflammatory bowel disease was similar to that in children without inflammatory bowel disease. The prevalence in both groups was 0.6%. However, larger studies may be needed to confirm this finding in children.

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Appendix 1: Disease Activity Indices
 a)   PCDAI (0-100)

History (Recall, 1 week) Abdominal pain:	None	0
	Mild - Brief, does not interfere with activities	5
	Mod/severe - daily, longer lasting, affects activities, nocturnal	10
Stools (per day):	0-1 liquid stools, no blood	0
	Up to 2 semi-formed with small blood, or 2-5 liquid	5
	Gross bleeding, or $\geq$ 6 liquid, or nocturnal diarrhea	10
Patient Functioning, General Well-Being (Recall, 1 week):	No limitation of activities, well	0
	Occasional difficulty in maintaining age appropriate activities	5
	Frequent limitation of activity, very poor	10
Laboratory	HCT (%)<10 yrs: >33	0
	11-14M: $\geq$ 35	0
	28-32	2.5
	30-34	2.5
	< 28	5
	< 30	5
	11-19F: $\geq$ 34	0
	15-19M: $\geq$ 37	0
	29-33	2.5
	32-36	2.5
	< 29	5
	< 32	5
	ESR (mm/hr) < 20	0
	20-50	2.5
	> 50	5
	Albumin (g/dL) $\geq$ 3.5	0
	3.1-3.4	5
	$\leq$ 3.0	10

PCDAI (Cont.)			
Examination	Weight	Weight gain or voluntary weight stable/loss	0
		Involuntary weight stable, weight loss 1-9%	5
		Weight loss 10%	10
	Height at Diagnosis	<1 channel decrease	0
		1 to <2 channel decrease	5
		>2 channel decrease	10
	Follow-up Height velocity	-1 SD	0
		<-1SD, >-2SD	5
		-2SD	10
	Abdomen	No tenderness, no mass	0
		Tenderness, or mass without tenderness	5
		Tenderness, involuntary guarding, definite mass	10
	Perirectal disease	None, asymptomatic tags	0
		1-2 indolent fistula, scant drainage, no tenderness	5
		Active fistula, drainage, tenderness, or abscess	10
	Extra-intestinal Manifestations Fever =38.5 for 3 days over past week, definite arthritis, uveitis, E. nodosum, P. gangrenosum	None	0
		One	5
		Two	10

PUCAI (0-85)

1) Abdominal Pain	No Pain	0
	Pain can be ignored	5
	Pain can not be ignored	10
2) Rectal bleeding	None	0
	Small amount in less than 50% of stools	10
	Small amount with most stools	20
	Large amount (>50% of stool content)	30
3) Stool consistency of most stools	Formed	0
	Partially formed	5
	Completely unformed	10
4) Number of stools/24 hours	0-2	0
	3-5	5
	6-8	10
	> 8	15
5) Nocturnal stools (any episodes causing waking	No	0
	Yes	10
6) Activity level	No limitation	0
	Occasional limitation	5
	Severe restricted activity	10

Appendix 2:

Child Information Sheet (Patients and Controls)

Title:

Celiac disease in children with inflammatory bowel disease

Investigator(s):

Principal Investigator: Dr Wael El-Matary 7804073339

Co-investigator: Dr Hien Huynh 7804073339

Co-investigator: Dr. Justine Turner 7804073339

Co-investigator: Dr Rabin Persad 7804073339

Purpose of the research:

Some patients with or without colitis may have another bowel disease called celiac disease we are doing this study to see if your child have got celiac disease or not.

This will help us to optimize his/her medical care and well being.

Description of the research:

We are recruiting with and without colitis to see weather celiac disease is more common in those with colitis compared to those without. As part of this study, we will record details of your child's symptoms (such as bloody diarrhea and abdominal pain) and results of routine blood tests. We might ask you some questions. As part of this study, some additional blood testing is required. This extra blood (5ml, 1-2 teaspoons) will be collected only at the time of a regular blood test when you come to the clinic. If you child's blood proven positive for celiac disease, you will be informed, and as part of our routine care in these situations, we will need to confirm that by doing endoscopy and small bowel biopsies. If celiac disease is proven, your child will need to avoid certain kinds of food.

The therapy your child receives will NOT be influenced by this study. Your child will NOT be asked to stay longer in the hospital or to return to the clinic as part of the study.

Potential Harms:

We know of no harm that taking part in this study could cause.

Potential Discomforts or Inconvenience:

Answering our questions may involve some inconvenience, but this takes about 10 minutes. Some extra blood is required for testing, but this will be taken at the time of regular blood testing.

Potential benefits:

Your child may benefit from participation in this study. If your child has celiac disease, he/she will need to avoid certain kinds of food. Patients with celiac disease may have health problems if they do not avoid certain kinds of food (wheat, rye and oat)

Confidentiality:

Personal records relating to this study will be kept confidential. Any research data collected about your child during this study will not identify him/her by name, only by initials and a coded number. Your child's name will not be disclosed outside the research clinic. Any report published as a result of this study will not identify you by name.

For this study, the study doctor may need to access your child's personal health records for health information such as past medical history and test results. He/She may also need to contact your child's family physician and his/her other health care providers to obtain additional medical information. The health information collected as part of this study will be kept confidential unless release is required by law, and will be used only for the propose of the research study. By signing the consent form you give permission to the study staff to access any personally identifiable health information which is under the custody of other health care professionals as deemed necessary for the conduct of the research.

The health information collected in this study will need to be checked from time to time against your medical records by the investigators or the Health Research Ethics Board may have access to your child's records to monitor the research and verify the accuracy of study data.

By signing the consent form you give permission for the collection, use and disclosure of your medical records. The data produced from this study will be stored in a secure, locked location for 7 years. Even if you withdraw from the study, the medical information which is obtained from your child for study purposes will not be destroyed. You have a right to check your health records and request changes if your personal information is incorrect.

Voluntary participation:

You and your child are free to withdraw from the research study at any time, and your child's continuing medical care will not be affected in any way. If the study is not undertaken or if it is discontinued at any time, the quality of your child's medical care will not be affected. If any knowledge gained from this or any other study becomes available which could influence your decision to continue in the study, you will be promptly informed.

Compensation for injury:

If your child becomes ill or injured as a result of participating in this study, necessary medical treatment will be available at no additional cost to you. By signing this consent from you are not releasing the investigator(s) and institution(s) from their legal and professional responsibilities.

Contact names and telephone numbers:

If you have concerns about your rights as a study participant, you may contact the Patient Relations Office of Capital Health, at 780-407-0808

If you have any questions about this study, please call Dr Wael El-Matary at 780-407-3339.

Appendix 3:

Patients' Data Collection Sheet

Patient#_____   Initials_____   Date:

Date of birth YYYY-MM-DD

Diagnosis   CD   UC   IC

Date of diagnosis

Child's ethnicity   Caucasian   Eastern Europe   Western Europe   Black   Chinese   Jew

Asian   Middle East   Mixed   Hispanic   Native   Unknown

Father's ethnicity   Caucasian   Eastern Europe   Western Europe Black   Chinese   Jew

Asian   Middle East   Mixed   Hispanic   Native   Unknown

Mother's ethnicity Caucasian   Eastern Europe   Western Europe Black   Chinese   Jew   Asian

Middle East   Mixed   Hispanic   Native   Unknown

Disease location: Rectum   Distal colon   Pancolonic   TI   Ileocolon

                        Small bowel   Stomach   Esophagus   Panenteric   Perianal   Mouth

Current medications:

Any other current diseases

Diseases in the past

Family History of IBD   CD   UC   IC   Who?

How was the diagnosis made?   Colonoscopy   Medications:

Personal/+Family history of: IDDM   Y   N   Who?

Thyroid disease Y   N   What?   Who?

Parathyroid   Y   N   What?   Who?

Skin (vitiligo)   Y   N   What?   Who?

Joint (JCA)   Y   N   What?   Who?

Asthma   Y   N   Who?

Liver (autoimmune)   Y   N   Who?   MS   Y   N   Who?

TTG levels

IgA levels

How many OGDs

 Duodenal Bx   Y   N   Normal   Abnormal

TI Bx   Y   N   Normal   Abnormal

Small bowel histology:

Villous atrophy   Y   N

Intraepithelial lymphocytes   Y   N

Lymphocytic infiltration   Y   N

Appendix 4:

Controls Data Collection Sheet

Patient#_____ Initials_____   Date:

Date of birth YYYY-MM-DD

Major Compliant:

Child's ethnicity   Caucasian   Eastern Europe   Western Europe   Black   Chinese   Jew

Asian   Middle East   Mixed   Hispanic   Native   Unknown

Father's ethnicity   Caucasian   Eastern Europe   Western Europe Black   Chinese   Jew

Asian   Middle East   Mixed   Hispanic   Native   Unknown

Mother's ethnicity Caucasian   Eastern Europe   Western Europe Black   Chinese   Jew   Asian

Middle East   Mixed   Hispanic   Native   Unknown

Diagnosis

Date of diagnosis

Any current disease

Any chronic diseases in the past

TTG levels

IgA levels

Personal/Family History of IBD   CD   UC   IC   Who?

How was the diagnosis made?   Colonoscopy   Medications:

Family history of: IBD   CD   UC   ID   Who?

IDDM   Y   N   Who?

Thyroid disease   Y   N   What?   Who?

parathyroid   Y   N   What?   Who?

Skin (vitiligo)   Y   N   What?   Who?

Joint (JCA)   Y   N   What?   Who?

Asthma   Y   N   Who?

Notification Re-approval

Principal Investigator:	Wael El-Matary
Renewal ID:	Pro00002008_REN1
Study ID:	Pro00002008
Study Title:	Prevalence of celiac disease in children with inflammatory bowel disease.
Approval Expiry Date:	11/27/2009

Thank you for returning the request for re-approval of this study. The Health Research Ethics Board (Biomedical Panel) has reviewed the file on this project for which all documentation is currently up to date. The research has been found to be acceptable within the limitations of human experimentation.

Specific Comments:

The expiration date for this approval is noted above. A renewal report or closure notice must be submitted next year prior to the expiry of this approval. You will receive electronic reminders at 60, 30, 15 and 1 day(s) prior to the expiry date. If you do not renew on or before that date, you will have to submit a new ethics application.

For studies where investigators must obtain informed consent, signed copies of the consent form must be retained, as should all study related documents, so as to be available to the HREB on request. They should be kept for the duration of the project and for at least seven years following its completion. In the case of clinical trials approved under Division 5 of the Food and Drug regulations of Health Canada, study records must be retained for 25 years.

Yours sincerely,

S.K.M. Kimber, MD, FRCPC
Chair, Health Research Ethics Board (Biomedical Panel)
Note: This correspondence includes an electronic signature (validation and approval via an online system).