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Celiac Disease is Uncommon in Irritable Bowel Syndrome in the United States

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Abstract

Background & Aims—Routine serologic testing for celiac disease (CD) may be useful in IBS patients but this is controversial. We aimed to compare the prevalence of unrecognized CD in a large cohort of patients with and without IBS.

Methods—Case-control study from a family case-control IBS study at a single US academic medical center. Stored serum and DNA utilized. Tissue transglutaminase (TTg) IgA performed, followed by indirect immunofluorescence testing for endomysial antibodies with positive or weakly positive TTg results. Individuals considered having CD if both results positive. Chi-square and Fisher's exact tests were used to compare prevalence between the two groups.

Results—Serum studied from 533 cases and 531 controls. 80% of participants female, median age 50; 65% of cases and 0% controls met Rome criteria for IBS. Prior serological testing for CD had occurred in 142 (27%) cases and 13 (2%) controls, but none had CD on subsequent testing. Six (1.1%) cases versus five (0.9%) controls had positive or weakly positive TTg test. Six cases (1.1%) vs. three (0.6%) controls confirmed to have CD by EMA ($p=0.51$).

Conclusions—No difference in the prevalence of CD between patients with IBS and patients without IBS at a tertiary medical center were observed. Our findings do not support routine celiac serologic or genetic testing in patients with IBS in all US populations.

Keywords

celiac disease; irritable bowel syndrome; screening serology

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INTRODUCTION

Celiac disease (CD) is a chronic condition, affecting approximately 1% of the population [1, 2]. Celiac disease is one of the great mimics in internal medicine. Although many patients with CD are asymptomatic or minimally symptomatic, classically CD presents with diarrhea and weight loss, but it can also occur in patients who report constipation, bloating or gastroesophageal reflux, and in the obese [3, 4]. Gluten intolerance in CD leads to increased intraepithelial lymphocytes and may result in severe cases in complete villus atrophy [5]. Patients diagnosed with CD following a strict gluten free diet may find their symptoms resolved but intolerance to gluten is permanent [6].

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder in the United States affecting 15% of Americans [7]. Specifically, IBS is a complex syndrome consisting of abdominal pain accompanied with diarrhea (looser or more frequent bowel movements) and/or constipation (harder or less frequent bowel movements) [8]. Urgency, bloating, visible distention, straining, or incomplete evacuation may accompany the pain with altered bowel habits; these symptoms support the diagnosis but are not part of the current diagnostic Rome criteria [9].

Due to variability in symptoms, IBS has been further classified into predominant-symptom subtypes: constipation-predominant IBS (IBS-C), diarrhea-predominant IBS (IBS-D), and mixed constipation and diarrhea (IBS-M). Importantly, IBS-like symptoms occur in organic diseases including inflammatory bowel disease in remission, microscopic colitis and CD [10, 11]. An objective diagnostic test for IBS has yet to be identified but a positive diagnosis can be made based on the presence of typical symptoms although the role of diagnostic testing to exclude organic disease has experts divided [9, 12].

A systemic review by Cash et al evaluated the utility of diagnostic testing in IBS patients and showed insufficient evidence to recommend a routine standardized battery of tests in patients who meet symptom-based criteria for IBS in clinical practice guidelines aside from celiac disease testing [13, 14]. The current American College of Gastroenterology (ACG) Position Statement on irritable bowel syndrome does not advocate routine investigation unless there are alarm symptoms present but recommends routine serologic screening for CD in patients with IBS-D and IBS-M [10]. Similarly, ACG guidelines on CD suggest testing for CD in patients with chronic or recurrent diarrhea, abdominal distension or bloating with or without abdominal pain [15, 16]. On the other hand, Cash et al. in a case-control US study of 492 patients with symptoms of IBS and 458 asymptomatic individuals who underwent colonoscopy examinations for cancer screening or polyp surveillance (controls) observed 7% had abnormal results for CD-associated antibodies, compared with early 5% of controls, a non-significant difference [14]. Irvine et al. [17], in a meta-analysis of 36 studies found prevalence of positive serological test for CD among those with suspected IBS to be between 2.6 and 5.7% while a biopsy-proven prevalence of CD to be 3.3%.

We aimed to determine the prevalence of unrecognized CD in a large cohort of patients with and without IBS. To determine the yield of serological testing for CD, we performed

screening tissue transglutaminase IgA (TTg) testing on sera collected from all cases and controls who were enrolled in a large family case-control study. Confirmatory testing with endomysial antibody (EMA) testing was performed in the samples of those with positive or weakly positive TTg tests as previously validated by us [18].

In addition, genotyping to determine CD HLA risk status was performed. Our overall goal was to provide an evidence-based justification for--or against--celiac serologic and genetic testing in patients presenting with IBS. This information can be found in the Supplementary Material.

METHODS

Participants

The study sample consisted of cases with IBS and age-, gender-, race-, and residency region frequency-matched controls. Greater detail regarding the context of this multi-aim study has been published [19]. Cases were prospectively recruited outpatients who had been clinically diagnosed with IBS by a *GI physician, a direct referral by a treating physician, through recruitment at an IBS patient education class, or by using a research database of medical records at Mayo Clinic that is updated daily and was programmed to search for patients with IBS listed among their final diagnoses* between July 2004 and June 2007[20]. All cases with an alternate gastrointestinal diagnosis (*i.e. celiac sprue, inflammatory bowel disease, or major abdominal surgery*) on chart review were excluded from the study. Controls were prospectively recruited between February 2005 and July 2007 selected among patients who had been seen in the Division of General Medicine for a general medical examination or were identified via computer-based search of medical records of patients without IBS listed in their medical history, *with recruitment purposefully lagging behind cases to allow for frequency matching. Recruitment of case and control probands was performed in person if being seen in clinic or by mail if identified through the computerized database*[19]. Controls with an IBS diagnosis, reported an IBS diagnosis, or met Rome criteria I or II on the screening questionnaire were excluded. This study was approved by the Mayo Clinic Institutional Review Board (IRB).

Data Collection

Once consented, all cases and controls were asked to complete a study questionnaire, which included the validated Bowel Disease Questionnaire (BDQ) [21], and provided a 20 ml blood sample which was then centrifuged and separated into DNA and serum. An abstraction was conducted on the participants' medical chart to review for exclusionary criteria, prior symptoms and testing results.

Study Definitions

Predominant IBS subtypes were based on definitions outlined by Rome criteria [8]. We applied two different definitions:

Rome I was defined as at least 3 months of continuous or recurrent symptoms of abdominal pain or discomfort that is: 1) relieved with defecation; and/or 2) associated with a change in

frequency of stool; and/or 3) associated with a change in consistency of stool; and two or more of the following, at least on one fourth of occasions or days: 1) altered stool frequency; 2) altered stool form (lumpy/hard or loose/watery stool); 3) altered stool passage (straining, urgency, or feeling of incomplete evacuation); 4) passage of mucus; and/or 5) bloating or feeling of abdominal distension [22].

Rome II was defined as at least 12 weeks (which need not be consecutive,) in the preceding 12 months of abdominal discomfort or pain that has two out of three features: 1) relieved with defecation; and/or 2) onset associated with a change in frequency of stool; and/or 3) onset associated with a change in form (appearance) of stool [23].

Rome III (modified) was defined as at least 3 months of recurrent abdominal pain or discomfort associated with the following 1) onset associated with a change in frequency of stool and 2) onset associated with a change in form (appearance) of stool [12].

Constipation-predominant IBS (IBS-C) was defined as meeting Rome criteria for IBS and endorsing one or more constipation symptoms greater than 25% of the time (<3 bowel movements per week, hard stools, straining) and no diarrhea symptoms greater than 25% of the time (>3 bowel movements per day, loose stools, urgent stools).

Diarrhea-predominant IBS (IBS-D) was defined as meeting either Rome criteria, and endorsing 1 or more diarrhea symptoms but no constipation symptoms. Mixed-IBS (IBS-M) was defined as meeting Rome criteria for IBS but not meeting criteria for IBS-C or IBS-D.

Family History

IBS family history was based on data collected from a participating first-degree relative, for relatives who did not participate, proband-provided information was used to assign IBS status for that specific relative. To be considered having IBS by self-report, the relative must have reported an IBS diagnosis and/or met Rome I or II criteria for IBS, and not reported an alternative gastrointestinal condition such as inflammatory bowel disease or CD from a list of medical diagnoses. Celiac family history was based on data collected directly from participating relatives and was defined as a positive endorsement of a diagnosis of CD on the medical history list.

Procedures

CD serological testing—Tissue transglutaminase immunoglobulin A (TTg IgA) antibodies were measured using a commercially available kit using recombinant human tissue transglutaminase as a substrate (INOVA Diagnostics, Inc. San Diego, CA) [24]. Each sample was diluted 1:101 by adding 5 µl of serum to 500 µl of histidine-rich protein (HRP diluent). Diluted samples were tested within 8 hours of preparation. Lab positive and negative control samples were pre-diluted. Average obtained absorbance for each sample was calculated and then converted to an ELISA value by reference to the 5-part point calibration curve. Result interpretation was as follows: negative, <4.0 U/ml; weak positive, 4–10 U/ml; and positive, >10 U/ml.

Endomysial antibodies (EMA) using immunohistochemistry was performed in those with positive or weakly positive TTg test results [25]. Diluted serum samples were incubated with air-dried 5- μ m cryostat sections of monkey esophagus and unbound antibody was rinsed free. Substrate-bound IgA antibody was identified with fluorescein-conjugated antihuman IgA (Fab₂-specific) conjugate, and the staining pattern examined by microscope for epifluorescence. Positive staining was identified as a reticulated lacelike pattern on perimucosal smooth muscle bands at dilutions of 1:5 or greater. Sera positive for endomysial antibody were titrated to end point by doubling dilutions.

Individuals were considered to have CD if both TTg IgA and endomysial antibody tests were positive [26, 27]. We have shown specificity is maximized by the use of the endomysial antibody test [18].

Statistical Analysis

Continuous data was compared using a Kruskal-Wallis test. Chi-square and Fisher's exact test was used to compare the prevalence of positive serologic testing between the cases and controls. Diagnostic specificity was evaluated against the disease control group.

SAS statistical software (SAS Institute Inc., Cary, NC, USA) was utilized for analyses. A p-value <0.05 was the threshold considered to be statistically significant.

RESULTS

Sample Characteristics

Demographic characteristics of the cases and controls are shown in Table 1. Of the 1064 total participants, median age was 50 (range 18–71) years with 847 (80%) females.

Yield of Celiac Testing

Results of celiac serological testing in our sample are shown in Table 2. Among the 1064 samples tested, the TTg test was positive or weakly positive in 11 (1.1%) and negative in 1053 (99.0%). Among cases, 6 (1.1%) were positive or weakly positive, while among controls, 5 (0.9%) were positive or weakly positive ($p=0.77$). Among the 11 individuals who subsequently underwent EMA testing, 9 (82% of 11) were EMA positive. By case-control status, 6 (1.1% of 533) were positive among cases and 3 (0.6% of 531) were positive among controls ($p=0.51$) (Table 2).

Medical chart review revealed 142 (27%) of the cases and 13 (2%) of the controls had prior serological testing for CD, but none were found to have CD with subsequent testing. When analysis was limited to cases and controls without prior testing, no difference was observed in the prevalence of CD between test-naïve cases and controls (1.5% v. 0.6%, $p=0.18$).

When analysis was limited to cases and controls from the local region, 2 (1.2%) of the cases and 1 (0.4%) of the controls had CD by testing, which was not different between the groups ($p=0.57$). When analysis was restricted to cases meeting Rome criteria for IBS, the proportion of those with serological findings consistent with CD was still 1.2% vs. 0.6% of controls ($p=0.44$).

Characteristics of participants testing positive for CD

Table 3 summarizes features of the nine individuals who tested positive for CD. Of the six IBS cases serologically positive for CD, four were female while two were male, and came from all geographic regions of the U.S. Four of the six cases met Rome I or II criteria for IBS, met criteria for either IBS-M or IBS-C (not IBS-D), and self-described their recent bowel habits as either normal, mixed, constipated, or “other”. IBS diagnosis or symptom onset ranged from 24 to 45 years of age. Three had another family member with IBS based on proband report or self-report by a participating first-degree relative, while none had another family member with known CD. Clinical evaluation was offered to the nine participants with positive serology, and three of the participants had a duodenal mucosal biopsy to confirm the diagnosis. Additional information can be found in the Supplementary Material.

DISCUSSION

This study from an academic medical center found, upon screening stored serum from patients with a clinical diagnosis of IBS and patients without IBS via the sensitive TTg IgA testing followed by specific EMA testing, a diagnosis of CD was uncommon in both IBS cases and controls without IBS. We observed serologic CD in 1.1% of cases with a diagnosis of IBS and 0.6% of controls. This difference between cases and controls was not significantly different. Our data are consistent with another US case-control study (n=950) where CD was confirmed histologically in 0.41% of IBS cases without constipation and similarly in 0.44% of colonoscopy controls [14].

Notably, we did not observe the over 4-fold higher risk of CD in patients with IBS cited by the systematic review by Cash et al. [13] and Ford et al. [28]. Sanders et al. conducted an influential study in the U.K.; the investigators observed 4% of cases vs. 0.7% controls had positive EMA tests. Most recently, Irvine et al. [17] found the prevalence of positive celiac serology and biopsy-proven CD to be significantly higher amongst subjects with suggestive symptoms of IBS rather than healthy controls in a large meta-analysis. When we restricted our sample to local patients or patients without prior testing, the highest prevalence of CD observed was still only 1.5%. This difference in study findings may be explained by a lower prevalence of CD in the U.S. compared with the U.K. but most of our IBS cohort was Caucasian. There have been differences in the selection of patients in practice for referral for evaluation for IBS and this might account for the variable results [29–31]. Sample sizes, racial backgrounds and screening methods for CD varied from study to study, and of the three studies ultimately reporting EMA test results [32–34], the prevalence of positive EMA results in IBS cases was 4%, 0.5%, and 0%, respectively. Our study findings fall into the lower range of what has been reported by other investigators.

Among the patients found to have undiagnosed CD, we observed bowel habits and clinical presentation were heterogeneous. This finding is not surprising in light of reports by others demonstrating CD presentation is quite variable [35]. Interestingly, family history of CD or IBS was not common in those diagnosed with CD in this study. Only one case (with mixed bowel habits) had another family member diagnosed with IBS, seeming to suggest although CD is a genetic disorder which has been shown to aggregate in families with greater

concordance in monozygotic twins than dizygotic twins and linked to HLA-type, family history is not a reliable marker for disease. One control did report a mother with CD. Similarly, various studies have found the role of family history in CD is inconsistent [3, 36].

Features which may affect the interpretation and generalizability of this study bear further discussion. First, this study was not a population-based study, but rather represented a referral population including local, regional, and national patients. A population-based study in a random sample of Olmsted County residents by Locke et al. reported the prevalence of TTg positivity was 4% among patients meeting Rome I criteria for IBS and 2.6% among asymptomatic controls, but none of these individuals had positive EMA test results, suggesting CD was relatively rare in the community at the time (1992–1995) [37]. A recent Olmsted County study found the overall incidence of CD between the years 2008–2010 to be 17.4 per 100,000 persons per year, one of the highest figures reported [38]. As many with IBS do not consult, a gap in knowledge regarding the yield in health-seeking individuals exists [39].

An additional limitation is patients with IBS recruited for this study were not incident cases. Because IBS symptoms may have a gradual onset, are not specific, and are not necessarily worrisome to warrant medical attention, true “incident” cases are difficult to capture. When we restricted the analysis to cases that did not have prior testing for CD, our conclusions did not change. We did not require IBS cases to meet standardized Rome criteria for IBS. Restricting the analysis to the majority of cases who did meet Rome criteria for IBS did not alter conclusions. While we did apply the Rome III criteria retrospectively, a potential limitation, the Rome II criteria provide similar diagnostic sensitivity and specificity [40]. *Additionally, our data is from a case-control study designed for other purposes. As a result, our sample size should have included 768 subjects per group to demonstrate CD prevalence in IBS subjects is the same in the general population.* Confirmatory biopsy—gold standard for CD diagnosis—was not available on six of nine participants with positive serology. Clinical evaluation was offered to the nine participants with positive serology, and three of the participants had a duodenal mucosal biopsy to confirm the diagnosis. Nonetheless, overall specificity of EMA testing makes false-positives highly unlikely [18, 41]. *Finally, we may have missed seronegative CD as IgA levels were not measured in this study. Immunoglobulin A deficiency has been reported in 2.5% of celiac disease and is commonly associated with CD than in the general population* [42, 43].

We acknowledge the applicability of our study findings will need to be reproduced in other centers and the generalizability of our findings to other patient populations will vary depending on individual facilities’ patient demographics. Our sample was predominantly Caucasian, and thus, results cannot be applied to non-Caucasian patients. Our study findings are important as it has been suggested serologic testing for CD may be useful in patients who meet Rome criteria for IBS in order to confidently make an IBS diagnosis [44]. Cost-effectiveness analysis of testing for CD (serologic testing followed by endoscopic biopsy for positive tests) in IBS-D patients has been performed by Spiegel et al., found testing for CD has an acceptable cost when the prevalence of CD exceeds 1% and is the dominant strategy when prevalence exceeds 8% [45]. Based on this, our findings suggest although CD can present with heterogeneous symptoms including IBS, serological testing may still be cost-

effective among US Caucasian patients with IBS but is not the dominant strategy. Other investigators have argued in addition to biopsy-proven CD, some individuals with gastrointestinal symptoms may have gluten-sensitivity which in some is the result of latent or potential CD, and this group was not addressed in the current research [46].

In conclusion, a large study of over 500 cases with IBS and over 500 controls, we found CD uncommon among patients with IBS compared to matched controls. There was a low prevalence of CD among those with diarrhea and in our varied patient populations. Among individuals diagnosed with CD, symptoms and family history were variable including many reporting normal bowel habits. We suggest serological evaluation (tissue transglutaminase IgA followed by endomysial antibodies) has a low yield to diagnose CD in US patients with IBS symptoms and cannot be routinely recommended.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Patient Sample Characteristics

	Cases	Controls
Number, n	533	531
Female, n (%)	439 (82%)	408 (77%)
Median age (range)	49.5 (18.0, 70.0)	49.9 (19.1, 70.7)
Residential region, n (%)		
Local (7 county region)	170 (32%)	242 (46%)
Regional (rest of MN, WI, IA, ND, SD)	192 (36%)	146 (27%)
National (rest of U.S.)	171 (32%)	143 (27%)
Race, n (%)		
Caucasian	522 (98%)	516 (97%)
Non-Caucasian	10 (2%)	15 (3%)
Unknown	1 (0%)	0 (0%)
IBS subtype, n (%)		
Rome criteria for IBS	349 (65%)	-
Rome I	335 (67%)	-
Rome II	297 (60%)	-
Rome III (Modified)		
IBS-C	56 (16%)	-
IBS-D	129 (37%)	-
IBS-M	155 (45%)	-
Other (non-Rome)	184 (35%)	-
Functional constipation	35 (19%)	-
Functional diarrhea	4 (2%)	-
Unspecified functional bowel disorder	76 (42%)	-

Table 2*Tissue* Transglutaminase (TTg) and Endomysial antibody (EMA) Results

	TTg		EMA	
	Cases	Controls	Cases	Controls
Positive	6 (1.1%)	5 (0.9%)	6 (1.1%)	3 (0.6%)
Negative	527 (98.9%)	526 (99.1%)	527 (98.9%)	528 (99.4%)

Table 3

Participant characteristics with positive EMA results

Case/Control Status	Gender	Age	Region ¹	Rome Status	IBS subtype	Bowel habit <30d	Age IBS diagnosis	IBS family history	Celiac family history	HLA-DQ2.5 result	HLA-DQ8 result
Case	Male	51	3	Neg	N.A.	Normal	49 ²	No	No	Yes	No
Case	Male	66	2	Neg	N.A.	Other	41	Yes	No	Yes	No
Case ³	Female	24	1	Pos	IBS-M	Mixed	24	No	No	Yes	No
Case ³	Female	43	2	Pos	IBS-C	Mixed	43	Yes	No	Yes	No
Case ³	Female	51	1	Pos	IBS-M	Constip	45	No	No	Yes	No
Case	Female	69	2	Pos	Cannot determine	Constip	31	Yes	No	No	Yes
Control	Male	45	2	Neg	-	Normal	-	No	No	Yes	No
Control	Female	33	1	Neg	-	Normal	-	No	Yes	Yes	No
Control	Female	64	2	Neg	-	Normal	-	Yes	No	Yes	No

¹ Region: 1=local, 2=regional, 3=national

² Age of IBS symptom onset

³ Small bowel biopsy-confirmed celiac

IBS, Irritable bowel syndrome; Neg, Negative; Pos, Positive, N.A., Not applicable; IBS-M, Irritable bowel syndrome-mixed, IBS-C, Irritable bowel syndrome-constipation, Constip, Constipation