

Screening for Celiac Disease in a North American Population: Sequential Serology and Gastrointestinal Symptoms

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OBJECTIVES: The prevalence of diagnosed celiac disease is <1 in 2,000 in the United States, but screening studies undertaken in European and other populations have revealed a much higher prevalence. The objective of this study was to determine the prevalence of celiac disease and the utility of screening in the general adult population of a geographically isolated area.

METHODS: Serum tissue transglutaminase antibodies (tTG-IgA) were measured in volunteer health-care participants aged ≥18 years at the annual Casper, Wyoming, Blue Envelope Health Fair blood draw. Subjects with positive tTG-IgA tests had their endomysial IgA antibodies checked. Double positives were offered endoscopy with small bowel biopsy. All subjects completed a short gastrointestinal (GI) symptom questionnaire.

RESULTS: A total of 3,850 residents of the Natrona County had serologic evaluation for celiac disease, 34 of whom tested positive for both tTG and endomysial antibody (EMA) IgA. Excluding three individuals with previous diagnosis of celiac disease, the overall prevalence of positive celiac serology in this community sample was 0.8%. All 31 subjects were offered a small bowel biopsy. Of the 18 biopsied subjects, 17 (94%) had at least partial villous atrophy. Symptoms that were reported by the fair attendees did not predict positivity.

CONCLUSIONS: Screening for celiac disease was widely accepted in this preventative health-care setting. Undiagnosed celiac disease affects 1 in 126 individuals in this Wyoming community. Most were asymptomatic or had atypical presentations. Serologic testing can readily detect this disease in a general population.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at <http://www.nature.com/ajg>

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INTRODUCTION

The prevalence of “suspected” celiac disease in Western nations varies from 1 in 87 to 1 in 500 individuals (1–5). The prevalence of diagnosed celiac disease is much lower, however, being somewhere between 1 in 500 and 1 in 9,000 individuals (6–8). In the past, diagnosis has been made more frequent in patients with classic symptoms, such as diarrhea, steatorrhea, weight loss or failure to thrive, and multiple vitamin deficiencies (9). Increasing awareness of the condition and availability of serologic tests have led to augmented case finding (10–12), with a corresponding change in

the types of presentations that lead to the diagnosis. Consequently, an increasing proportion of patients without classic features of malabsorption or diarrheal illness have been identified (8,13).

For many years, celiac disease has been considered rare in the United States, although an increased frequency of celiac disease was being reported in European countries (14). Recently a large multicenter study in the United States, which included a substantial cohort of subjects regarded as “healthy,” suggested a celiac disease prevalence of 1 in 133 (15). However, there has been much debate about the most efficient strategy for detecting these

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additional celiac disease cases (16,17). If physicians only test those with severe, classic, and malabsorptive symptoms, the patients with atypical symptoms, who make up the majority of cases, would be missed; even testing those in whom celiac disease is more likely to occur (e.g., those with an affected family member, iron-deficient anemia, type 1 diabetes, or premature osteoporosis) will miss most cases (18). Other than some well-defined clinical associations, little is known about the symptoms that may provide clues to early diagnosis (9,19). In this regard, it has been suggested that the presence of symptoms suggestive of irritable bowel syndrome may predict celiac disease (20–23), but this has remained controversial. However, based on cost–benefit considerations and lack of an ideal screening strategy for celiac disease, to date a case-finding approach rather than population screening has been advocated by others (12,24).

The aims of this study were to determine the prevalence of undiagnosed celiac disease in a large population of adults in a geographically predefined area, and to identify any symptoms that predict celiac disease. Our secondary aim was to determine the efficiency and acceptability of screening for celiac disease in a health fair setting.

METHODS

Study design

Annual health fairs have been a longstanding practice and a traditional mode of providing preventative health care and information in Wyoming. This practice grew out of the seminal studies on community-based testing and treatment of streptococcal infection in the 1950s (25). Traditionally, there has been wide participation of the adult population in these fairs, which provide information regarding disease-specific prevention and low-cost tests for health screening purposes. This study took advantage of the well-established health fair in Natrona County, Wyoming. This county is a mixed urban and rural community of ~66,500 individuals (~49,000 adults) that is 91% Caucasian with the rest predominantly Hispanic or American Indian (US Census 2000). This site was chosen for several reasons. First, it is geographically isolated, with virtually all hospital-based care being delivered by the Wyoming Medical Center. Second, there is a single gastrointestinal (GI) practice that provides most of the gastrointestinal consultation and endoscopy services for the area, allowing prevalent cases of celiac disease to be readily enumerated. Last, there is a very active support group available for the patients with celiac disease in the area.

The laboratory component of the Health Fairs consisted of offering routine screening blood tests to individuals on an appointment basis. Individual participants select from a menu of tests. Typically, these tests include prostate-specific antigen, cholesterol, homocysteine, hemoglobin and a full blood count, liver chemistries, and measures of iron stores.

Subject recruitment

Individuals participating in the laboratory component of the health fair 2003 were invited to participate in this study when

they presented for a blood test. This strategy avoided recruiting individuals with a predetermined interest in testing for celiac disease. Each subject was given a short GI symptom questionnaire and a consent form on arrival at the blood draw site. Consenting subjects completed the questionnaire and had their serum saved for serologic examination. All participating individuals were informed about their test results. Subjects with previous history of celiac disease and those with serious medical problems at high risk for invasive procedures were excluded from further evaluation and statistical analysis. The study procedures were approved by the institutional review boards of the Wyoming Medical Center and the Mayo Foundation.

Questionnaire

The questionnaire used in the study was adapted from the internationally validated Nepean Dyspepsia Index, which is designed to be a short, rapidly completed questionnaire suitable for general population surveys (26). The questionnaire asked subjects to report if they experienced upper GI (a full feeling after meals, food stays in stomach, nausea, vomiting, heartburn, dysphagia) or lower GI (diarrhea, >3 bowel movements/day, watery, urgent, or incontinent stools, constipation, <3 bowel movements/week, hard stools, feeling of anal blockage, bloating) symptoms, and to rate the frequency and severity of any symptoms from 1 to 5 (rare and mild to frequent and severe). Each symptom was treated as a separate parameter in order to determine the clinically relevant severity.

Serological screening

Serum samples were shipped overnight to the testing laboratory in Rochester, Minnesota. A two-step sequential serologic testing strategy was used. First, the samples were tested for tissue transglutaminase IgA antibodies (tTG-IgA) using a commercially available enzyme-linked immunosorbent assay kit that used human recombinant tissue transglutaminase as the substrate (INOVA, San Diego, CA) as described in detail previously (21). Any test results in the positive or indeterminate range underwent subsequent testing with endomysial indirect immunofluorescence assay using monkey esophagus as the substrate at two screening dilutions (1:5 and 1:20) as described elsewhere (27).

Biopsy confirmation

All individuals who were positive, both on primary tTG and subsequently on secondary endomysial IgA testing, were informed of their results and, if there were no contraindications, they were offered endoscopic biopsies. Those consenting to undergo endoscopy had six distal duodenal biopsies obtained for histology. Pathologists in Wyoming who were not aware of the serologic results interpreted the biopsies. Pathological results were expressed in terms of architectural changes and inflammation as well as Marsh classification (28).

Statistical analysis

Prevalence rates for positive serology are expressed as percentages, accompanied by 95% exact (binomial) confidence intervals.

The distribution of categorical variables, such as the patient-reported symptoms, is summarized as counts and percentages. For laboratory parameters and other continuous measures, means and standard deviations (s.d.) are provided for variables that are approximately normally distributed, or medians and interquartile ranges otherwise. The descriptive statistics reported for all variables included the number of subjects with missing data. Patient-reported symptoms, originally captured on a five-point scale, were characterized in the analysis as present for responses of “sometimes,” “often,” or “very often,” and absent for responses of “never” or “rarely” to reflect the likely clinical relevance of the response for each symptom. Logistic regression was used to test for association between serology status and individual symptoms or laboratory parameters, controlling for age and gender. From these models, odds ratios and 95% confidence intervals are presented to convey the strength of association between each factor and having positive serology, independent of age and gender. A *P* value of <0.05 was considered statistically significant. Analyses were carried out using the SAS statistical software package (version 8.2, SAS Institute, Cary, NC).

RESULTS

A total of 82% (4,131 of 5,037) of health fair participants volunteered for this study. Of these, 3,850 lived in Natrona County and were included in the analysis. The ethnic composition of the cohort was predominantly Caucasian (94% Caucasian, 1% African American, 1% Native American, <1% Asian, <1% Pacific Islander, 2% from other races, and 2% from two or more races).

Prevalence estimate

The 3,850 Natrona County residents who underwent serologic testing for tTG IgA in their serum formed the denominator used for analysis. In all, 75 subjects were positive for tTG assay, of whom 34 subjects had positive sequential serologic testing (Figure 1). However, three seropositive subjects had a previous diagnosis of celiac disease and were consequently excluded from the estimate of undiagnosed celiac disease. Two seronegative subjects had a previous diagnosis of celiac disease.

Overall, the prevalence of undiagnosed celiac disease based on positive tTG and endomysial antibody (EMA) tests in this population-based sample in Natrona County was 0.8% (95% confidence interval 0.6–1.1%). The mean age (\pm s.d.) of 54.5 (\pm 14.7) years in the seropositive subjects was not statistically different from that in the seronegative group (57.5 \pm 14.7 years, *P*=0.23), although the numbers in the extreme age groups were small (Table 1). Of the 31 seropositive subjects, 65% (*n*=20) were female, compared with 59% among the seronegative population (*P*=0.55).

All individuals who were double positive and had no previous diagnosis of celiac disease were offered biopsies except for three subjects not felt to be medically fit for intervention due to their conditions (ischemic heart disease, heart failure, and metastatic lung cancer). Of the 18 subjects who underwent intestinal biopsy, 17 demonstrated at least partial villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis. In all, eight subjects had Marsh 3c, 6 had Marsh 3b, and 3 had Marsh 3a. The one subject with a negative duodenal biopsy was Asian, and his tTG-IgA and EMA tests were both weakly positive. This latter subject's included one biopsy with Brunner glands suggesting that the bulb had been

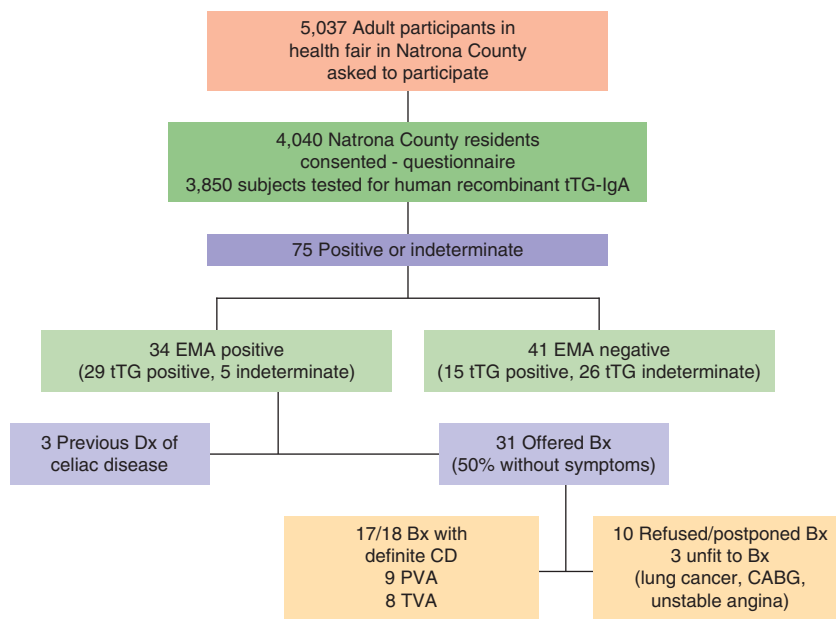


Figure 1. Study flowchart. Bx, biopsy; CABG, coronary artery bypass graft; CD, celiac disease; Dx, diagnosis; EMA, endomysial antibody; PVA, partial villous atrophy, tTG, tissue transglutaminase; TVA, total villous atrophy.

Table 1. Prevalence of undiagnosed celiac disease by age group

Age group	No. of positive/ group	Proportion % (95% confidence interval)
18–30	2/130	1.5 (0.2–5.5)
30–40	2/260	0.8 (0.1–2.8)
40–50	7/730	1.0 (0.4–2.0)
50–60	8/998	0.8 (0.4–1.6)
60–70	6/844	0.7 (0.3–1.5)
70–80	5/698	0.7 (0.2–1.7)
80+	1/187	0.5 (0.0–3.0)
Overall	31/3,847	0.8 (0.6–1.1)

sampled. Based on these findings, the positive predictive value for biopsy-confirmed celiac disease of this sequential serologic testing strategy was 94% for celiac disease.

Disease prediction by symptoms

From logistic regression modeling, adjusting for age and gender, no symptom was found to be positively associated with seropositivity (**Table 2**). The most common symptoms reported by subjects with positive serology included feeling full (46%) and heartburn (40%). Of the symptomatic subjects who were offered intestinal biopsy, 11 complained of constipation, 5 reported diarrhea, and 2 had a family history of celiac disease. Conversely, 8 (44%) of the 18 subjects who underwent intestinal biopsy were asymptomatic.

Associated laboratory abnormalities

Among the 2,438 (63%) subjects who had hemoglobin tested, the mean (\pm s.d.) hemoglobin level in 23 seropositive subjects was 15.0 g/dl (\pm 1.4) compared with 15.2 g/dl (\pm 1.3) in 2,415 seronegative subjects ($P=0.38$ adjusted for age and gender). Three (13%) seropositive subjects had a hemoglobin value <13 g/dl compared with 104 (4%) seronegative subjects ($P=0.06$). All but 12 subjects were tested for total cholesterol, with a mean level of 198.9 (\pm 31.3) in the 31 seropositive subjects and 206.3 (\pm 38.9) in 3,804 seronegative subjects ($P=0.32$). Only 882 subjects (23%) underwent ferritin testing. Within this subgroup, 7 seropositive subjects had a median (interquartile range) ferritin level of 10.0 (8.0, 117.0) compared with a median of 53.0 (19.0, 145.0) in 875 seronegative subjects ($P=0.23$). Out of the 7 seropositive subjects, 5 (71%) had ferritin values <20 g/dl compared with 230 of 875 (26%) seronegative subjects ($P=0.037$). In addition, seropositive status was associated with lower levels of high-density lipoprotein and calcium, although the latter was not statistically significant ($P=0.02$ and 0.08, respectively; **Supplementary Table S1** online).

DISCUSSION

This study confirms that celiac disease is relatively common in the general population. This conclusion is further reinforced by

Table 2. Patient-reported symptoms by serology status

Symptom ^a	Negative (n=3,816)	Positive (n=31)	Logistic result ^b , OR (95% CI) [P value]
Feeling full	1,253 (34%) [180]	14 (47%) [1]	1.58 (0.76–3.29) [0.22]
Bloating	1,305 (37%) [249]	9 (30%) [1]	0.69 (0.31–1.52) [0.35]
Heartburn	1,463 (41%) [226]	12 (40%) [1]	0.94 (0.45–1.95) [0.86]
Nausea	501 (14%) [208]	4 (14%) [2]	0.87 (0.30–2.56) [0.80]
Vomiting	132 (4%) [274]	1 (4%) [3]	0.91 (0.12–6.77) [0.92]
Diarrhea	964 (27%) [223]	5 (17%) [1]	0.52 (0.20–1.38) [0.19]
Constipation	1,114 (31%) [239]	11 (38%) [2]	1.33 (0.61–2.89) [0.47]
>3 BMs a day	810 (23%) [253]	2 (7%) [1]	0.24 (0.06–1.01) [0.05]
<3 BMs a week	431 (12%) [296]	4 (13%) [1]	1.01 (0.35–2.96) [0.98]
Lumpy or hard BMs	1,137 (32%) [245]	10 (34%) [2]	1.09 (0.50–2.39) [0.82]
Watery BMs	870 (24%) [256]	5 (17%) [2]	0.63 (0.24–1.66) [0.35]
Urgent need to have BM	1,259 (35%) [242]	9 (31%) [2]	0.84 (0.38–1.85) [0.66]
Any symptoms	2,924 (81%) [216]	24 (80%) [1]	0.87 (0.35–2.17) [0.77]
Sum of symptoms ^c	3.0 \pm 2.6 [120]	2.9 \pm 2.8 [1]	0.96 (0.83–1.11) [0.56]
NDI symptom score ^c	28.6 \pm 8.7 [117]	27.8 \pm 9.7 [1]	0.98 (0.94–1.03) [0.46]

BM, bowel movement; CI, confidence interval; NDI, Nepean Dyspepsia Index; OR, odds ratio.
^aUnless indicated otherwise, count (%) [number of subjects with missing data] values provided.
^bAdjusted for age and gender.
^cMean \pm s.d. [number of subjects with missing data].

histological confirmation in the majority of individuals with positive celiac serology. However, individual GI symptoms did not identify the seropositive cases. Interestingly, despite a relative lack of awareness of celiac disease in the general population, there was a wide acceptance of screening for this condition among the health fair participants in our study.

One of the major concerns regarding screening protocols in the general population is the likelihood of false-positive results that may lead to unnecessary invasive evaluations; however, this potential problem was minimized here as the serologic strategy applied in our study took advantage of relatively sensitive anti-tTG-IgA testing (94%) followed by highly specific EMA

measurement (100%) (29). Our findings are consistent with previous studies on the prevalence of celiac disease that have demonstrated that screening for celiac disease with this sequential method is an effective method to detect undiagnosed cases (30,31). In our study, the positive predictive value of this sequential testing was acceptable, suggesting this method as an efficient way to carry out population-based screening, while minimizing the false positives. Due to the invasive nature of endoscopy, biopsy was exclusively offered to double-positive individuals. We acknowledge that because of the rigorous serologic standards required by this approach, we may have missed some individuals with otherwise undiagnosed celiac disease. In other words, even if a few cases of celiac disease were missed because of false-negative serologic test, those numbers are not likely to be great (31). Of course, the overall prevalence of previously undiagnosed celiac disease would be even higher if such patients were included. Although we have been able to achieve this level of sensitivity with this technique, a recent meta-analysis suggests a lower sensitivity of 89% for celiac disease serology (32). In addition, as tTG-IgG testing was not undertaken, the few subjects with IgA deficiency who could be completely seronegative might have been missed by this strategy. As such, this study may underestimate the true prevalence of celiac disease, although such a miss rate is minimal. Moreover, for the purpose of population-based screening, serology provides the only practical means of detecting the disease.

Is screening the general population justified? If we are to detect most patients with celiac disease, a community-based screening strategy such as this study could be applied. Celiac disease may meet the five WHO criteria used for determining the need for a screening program. First of all, it is relatively common. It can be detected by a noninvasive strategy with high sensitivity and specificity based on sequential serological testing. Celiac disease can be also detected at an early, often asymptomatic state, and is readily treated. Certain morbidities such as fractures are more frequent in celiac disease both before and following the diagnosis (33–36), but this increased risk is not seen in patients diagnosed as children (37). A recent study by Kurppa *et al.* (38) has showed that even subjects with normal villous structure, but a positive EMA, may suffer from complications of the disease and may benefit from a gluten-free diet.

Retrospective studies have been conducted using archival sera to diagnose celiac disease. In one study of 9,133 subjects using this same robust serological strategy, subjects with serologic evidence of undiagnosed celiac disease had a fourfold increased mortality compared with seronegative subjects (39). This is consistent with a large European study suggesting that subjects with positive anti-TTG antibodies are significantly at increased risk of cancer-related death (40). However a study from Finland, where the highest proportion of celiac disease is detected clinically, did not show an increase in mortality (41). Malignant complications of celiac disease—such as lymphoma or adenocarcinoma—may be the first presentation of celiac disease (42,43), but the increased risk usually rises later in life and is low in absolute terms.

This study addressed two main issues pertinent to screening for celiac disease. First, it demonstrated that despite the relative

rarity of the disease, most people approached were happy to undergo testing for celiac disease when participating in community-wide preventative health event. Second, it did not identify any simple GI symptoms that would predict those most likely to have celiac disease. The results of this study tell us that testing for celiac disease in the general population is a complex issue. On one hand, symptoms do not predict who will have celiac disease, suggesting that general population screening may be needed to find celiac disease. On the other hand, also tell us that the disease may have had little effect on the subjects. However, a recent study suggests that 30% of patients discovered by screening without apparent symptoms at the time of detection felt better after starting the gluten-free diet, although some had reduction in their self-perception of health (44).

This study has several limitations. It was conducted in a geographically isolated area with a predominately Caucasian population who may not be representative of the entire North American population and would probably have lower yields across non-Caucasian population. Nonetheless, as a mixed Caucasian population makes up the majority of subjects, it probably reflects the Caucasian US population that is most prone to celiac disease. Additionally, not all subjects who were serum positive were fit enough or agreed to have endoscopy. It was entirely the patient's decision not to undergo endoscopy and not, as was reported in a large previous study, because of refusal of insurance company coverage or unwillingness of the primary physician to order endoscopy (15). Furthermore, considering the costs associated with the serologic testing in routine diagnostic use and the number of subjects identified to have celiac disease, the cost effectiveness of this screening strategy is unclear. Conversely, the strengths of this study depend on the geographic isolation, the high acceptance rate among participants of the health fair, the collection of symptom data before testing using a standardized, validated questionnaire, and the lack of any previous advertising that might have biased participation by encouraging those particularly concerned about the possibility of celiac disease.

A complete long-term follow-up of the previously undiagnosed patients identified by celiac serologic screening is needed to further clarify the usefulness and benefit of population screening for celiac disease, as well as the associated impact on quality of life of the otherwise asymptomatic individuals. Considering the restrictive aspect of adherence to gluten-free diet, evaluation of the compliance with the treatment in the absence of typical symptoms for celiac disease would be the crucial component of such a study. We are now following these subjects regarding compliance with a gluten-free diet, the response to treatment, and the potential changes in laboratory parameters and mucosal histological features. In fact, we have observed that many of those who initially did not consent to biopsies did commence on a gluten-free diet (data not shown).

In conclusion, celiac disease is common in the US population. In general, symptoms do not appear to predict the occurrence of celiac disease in the population. Members of the general population are agreeable to serology screening and subsequent management but not all will agree to endoscopy. Screening of the general population can be accomplished with a high degree of specificity

using sequential serological testing; however, the who, when, and if screening of the general population are justified are still unanswered and require further study.

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CONFLICT OF INTEREST

Guarantor of the article: Joseph A. Murray, MD.

Specific author contributions: Kent D. Katz: conception, planning, study design, execution, collection and interpretation of data, overseeing the study, and drafting the article; Shahrooz Rashtak: conception, statistical analysis, interpretation of data, and drafting the article; Brian D. Lahr: statistical analysis; L. Joseph Melton III: study design, statistical analysis, and interpretation of data; Patricia K. Krause: laboratory testing; Kristine Maggi: data collection and overseeing the study; Nicholas J. Talley: study design; and Joseph A. Murray: conception, planning, study design, analysis and interpretation of data, laboratory testing, securing funding, and drafting the article. All authors have reviewed and approved the final draft submitted.

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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ It is suspected that a majority of people with celiac disease are undiagnosed.
- ✓ Undiagnosed celiac disease may be associated with increased long-term morbidity and mortality.
- ✓ Screening of the groups at increased risk of celiac disease will find a minority of those affected by celiac disease.
- ✓ Screening for undiagnosed celiac disease is controversial and has not been studied in a community-based cohort.

WHAT IS NEW HERE

- ✓ Screening for celiac disease is both feasible and readily accepted by members of the general population participating in a preventative health fair.
- ✓ The presence of common gastrointestinal symptoms does not predict celiac disease in the community.
- ✓ Serologic testing using sequential strategy (anti-tissue transglutaminase (tTG) followed by anti-endomysial antibody (EMA) provides an acceptable method for screening purposes that possesses high positive predictive value, thereby minimizing unnecessary interventions.

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