Original Article

Prevalence of Celiac Disease in Shiraz, Southern Iran

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ABSTRACT

Background/Aim: This study was performed to evaluate the prevalence of celiac disease (CD) in Shiraz, southern Iran. **Materials and Methods:** Serum samples were collected from 1440 persons (age range = 20-83 years, mean age = 45.4 years) in 2004 and screened for endomysial and tissue transglutaminase antibodies. A questionnaire was completed for all subjects in relation to gastrointestinal (GI) symptoms and cases with positive serology were requested to undergo small-bowel biopsy. **Results**: Seven cases (0.5%) were positive for IgA anti-tissue transglutaminase (anti-tTG), and only two (0.14%) were positive for IgA anti-endomysial antibody (anti-EMA), both of whom had highly positive anti-tTg levels (40.4 and 48.0 IU/l). The major clinical symptoms of CD, such as recurrent abdominal pain and change in bowel habits were present in all patients with positive anti-tTG assays. Only five subjects with positive serology agreed to undergo upper GI endoscopy and duodenal biopsy. Three of these cases were reported with Marsh I histologic findings, while in the two cases with positive serologic anti-EMA, more advanced forms of CD were present. **Conclusion:** The prevalence of CD in apparently healthy adults was lower than the reported series from northern parts of the country; therefore, we suggest a more long-term follow-up study in high-risk groups, especially in the apparently healthy subjects in our region.

Key Words: Anti-endomysial antibody (anti-EMA), anti-tissue transglutaminase antibody (anti-tTG), celiac disease, southern Iran

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Celiac disease (CD), also called gluten-sensitive enteropathy and nontropical sprue, is a common autoimmuine disorder characterized by an immune response to ingested gluten.[1] Until a few years ago, this disorder was considered to be almost exclusively affecting the people of European origin[2]; however, recent reports from other regions of the world revealed similar rates of disease when actively searched for the disease by serological tests.[3] Although the diagnosis is usually easy in patients affected by typical symptoms of the disorder such as growth failure, loss of weight, diarrhea, and deficiency of various nutrients, most patients suffer from only subtle, if any symptoms, leading to difficulty in diagnosis.[4] According to the risk of the population under study, the prevalence may differ significantly.[5-8] Some screening programs within healthy populations indicate that the disease is underdiagnosed in some areas;[9-11] however, others reported an overestimation.[5]

Until 1980s, CD was thought to be very rare in the Middle East[12-14] and was not considered as a possibility in the differential diagnosis of malabsorption syndromes.[15,16] The daily ingestion of wheat, rye, and barely results in long-term extraintestinal sequels in subjects with undiagnosed or untreated CD.[5,6] Therefore, the early detection of the disease and subsequent implications of gluten-free diet may be an appropriate method in prevention of its complications later in life.

To determine the prevalence of CD in our region, we screened the adult population in Shiraz, the largest city in southern Iran for presence of the disease by serological tests and histological study of small intestine.

MATERIALS AND METHODS

Subjects were randomly selected from the telephone directory of Shiraz in southern Iran with a population of around 1.5 million people, and were invited to participate in the study. The city was divided into 14 areas according to the first three digits of telephone number. Telephone number was randomly chosen from each area and 2000 individuals were invited to attend our study at Mottahari Gastroenterology Clinic affiliated to Shiraz University of Medical Sciences.

All subjects were of Iranian ancestry. The Ethics Committee of the university approved the study and an informed written consent was obtained from each individual participating in this study. Data on past medical history and drug therapies were obtained from all subjects using a questionnaire. Cases

with history of endocrine disorders, diabetes mellitus, liver disease, kidney dysfunction, collagen disease or autoimmune disease and those who were on hormone replacement or known drug therapies interfering with bowel function and affecting epithelium of intestines were excluded.

Study protocol

The study group was screened for anti-endomyosial (EMA) and tissue transglutaminase (tTG) antibodies in blood samples obtained in early 2004 and all subjects with a positive result were asked to undergo upper GI endoscopy. The last biopsy specimen was obtained in December 2004.

Serum antibody tests

After the exclusion of abovementioned cases, the serum samples from 1440 persons were simultaneously assessed in a blind fashion in two different laboratories. Tests for anti-EMA and anti-tTG antibodies were conducted, respectively, in laboratories of Department of Immunology and Gastroenterohepatology Research Center, Shiraz University of Medical Sciences. Serum IgA anti-tTG was evaluated by the enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Binding site, Minineph R, UK) and values ≥7 IU/l were considered as positive. Serum IgA class anti-EMA antibodies were evaluated by immunoflourescence assay (IFA). If the level of anti-tTG was <0.1 IU/ml, the total serum IgA level was measured for ruling out IgA deficiency.

Endoscopy

Upper GI endoscopy was performed using an Olympus Gastroscope, (GIF type Q20**)** at the Endoscopy Room of Department of Internal Medicine of Shiraz University of Medical Sciences by trained fellows or academic staff of the university. A pathologist obtained multiple duodenal biopsies for routine histological eosin studies. Histological evaluation was performed according to the Marsh classification and normal mucosa with <30 intraepithelial lymphocytes per 100 epithelial cells was considered as Marsh I, normal mucosa with more than 30 intraepithelial lymphocyte as Marsh II, and cases with villous atrophy as Marsh III.[17]

Statistical analysis

Statistical analysis was performed using SPSS software, version 11.5. Descriptive variables such as mean, median, and standard deviation were determined. One-way analysis of variance (ANOVA) was performed to find out the significance between low body mass index (BMI) in tTG-negative and tTG-positive groups. Chi-square (χ^2) test was performed to find out the association between anti-tTG positivism and GI symptoms.

RESULTS

Our study was performed on 1440 subjects with an age range of 29-83 years (mean = 45.3 years). Seven subjects (1:180) were positive for IgA anti-tTG antibody and only two (1:720) were positive for IgA-class anti-EMA antibody, both of whom had highly positive tTG levels: 48.8 and 40.4 IU/l. From these, 1433 subjects were negative for both tests, and no one had undetectable total serum levels of IgA class antibodies of <0.1 IU/ml [Table 1]. The mean weight and height in the negative anti-tTG group were 68 kg and 146.93 cm and in the positive anti-tTG group were 67.63 kg and 161.15 cm, while the differences were not statistically significant.

The mean BMI in the negative and positive groups was 26.13 and 25.02, respectively; however, the difference was not statistically significant. All the subjects with positive anti-tTG had a history of GI symptoms; however, none of them had a biopsy-confirmed CD in the first-degree relatives. Recurrent abdominal pain and intermittent diarrhea were present in almost all the patients with positive anti-tTG test.

The review of five subjects who agreed to undergo upper GI endoscopy and duodenal biopsy revealed Marsh I in three and Marsh II in two cases, while both of them had high titers of anti-tTG level in serum samples.

Table 1: Characteristics of cases with positive anti-tTG antibody test in a random population in Shiraz, southern Iran (2004)

Subjects	Gender (Male/Female)	Age (years)	Weight (kg)	Height (cm)	Anti-tTG titer	Anti -EMA	Endoscopic biopsy results	Classic GI symptoms of CD	Other
Case 1	M	44	78	184	40.4	+	-	+	Pervious endoscopy
Case 2	F	37	54	150	48	+	Mild inflammation	+	Pervious endoscopy
Case 3	F	25	52	150	9	-	Mild inflammation	+	
Case 4	M	24	53	177	7.3	-	-	+	
Case 5	M	69	64	165	8.7	-	Mildly increased lymphoplasma cell	+	
Case 6	M	70	70	150	8.9	-	-	+	
Case 7	F	32	61	157	12	-	Mildly increased lymphoplasma cell Villous hypertrophy	+	Pervious endoscopy

DISCUSSION

Celiac disease is the most common food intolerance in the world, involving genetically predisposed individuals consuming gluten-containing cereals in their diet. The disease is present not only in Europe, but also in population of European ancestry (North and south of America and Australia), North Africa, Middle East, and south of Asia, where until a few years ago, it was historically considered extremely rare.[2]

In a study on apparently healthy population of northern and southern Iran, the prevalence of gluten-sensitive enteropathy (GSE) in 2799 individuals with mean age of 33.7 years (range = 18-66 years) was 29 cases who were positive for IgA tTG-antibody, whereas only five were simultaneously positive for anti-EMA. Except in two subjects with normal small bowel histology, all other subjects had biopsy findings compatible with GSE, with a prevalence of 0.96% or 1:104.[18] Another study in 2000 in Iranian healthy blood donors (1580 males, 420 females; mean age = 35.5 years, range = 18-65 years) reported a prevalence of 0.006% (1/166).[12] In other studies from northern Iran, a high prevalence of CD was noticed in high-risk groups such as patients with chronic diarrhea and diabetes mellitus.[19,20] Although the reported series stated that the prevalence of CD was high in our country, few cases of CD were seen in our daily clinical practice, in spite of using newly developed serological tests such as anti-tTG that are available in most laboratories. Despite the application of simple serological tests in diagnosis of CD, there are few studies from Middle East countries addressing the prevalence of this disease in populations such as those in Syria and Israel.[14,21]

Most of the reported cases of CD in India are from the northern part where wheat products are the staple diet. North Indians are possibly genetically prone to develop this disease.[22-24] In a recent study conducted on 2500 healthy blood donors in Tunisia, seven cases (1:355, 0.028%) of CD were reported.[25]

The newly developed ELISA tests for IgA anti-tTG antibodies are now widely available and are easier to perform and also less expensive than the immunofluorescence assay that is used to detect IgA anti-EMA antibodies. Anti-tTG antibodies are highly sensitive (95%) and specific (94%) for the diagnosis of CD.[26,27] The diagnostic accuracy of anti-tTG immunoassays has been improved further by the use of human tTG instead of the nonhuman tTG preparations used in earlier immunoassay kits and up to 100% positive predictive value was also reported.[28,29]

In a recent study from our country, the sensitivity and specificity of tests were 100% and 99% for the human-recombinant IgA tTG-antibody assays, respectively, whereas only in cases with positive IgA EMA, advanced mucosal lesions of the small bowel were detected in intestinal biopsy. They concluded that in a general population, the best screening test for detection of GSE is IgA tTG-antibody.[18] Therefore, most anti-tTG positive cases in recently published reports from Iran had low titers in serologic testing and Marsh III stage in histological evaluation. Some authors considered this rate of CD in Iran to be an overestimation and suggested a follow-up study of the cases.[30] We used the human type IgA class serum anti-EMA antibody test, which has also been validated in Europe, to identify untreated CD cases.[31] In our study, the cases with high titers of anti-tTG had positive anti-EMA and more advanced histological findings. This population-based study showed that the prevalence of CD in our region may not be similar to the prevalence in the so-called western countries or Europe, and it is also different from that reported by other studies in northern and other regions of Iran. However, if we base the diagnosis of CD on positive anti-tTG and anti-EMA associated with Marsh II or III histology, the prevalence of CD in our country will not be as high as those reported figures. Thus, we should reconsider our definition of CD because the economical burden of screening for even mild forms of disease will be high. Another important fact that should be considered will be the effects of dietary habits in different regions of the country. Rostami *et al.*[16] stated that agriculture of wheat that was started from modern Turkey, Iraq and Iran, slowly spreading to Europe, and showed that wheat consumption had a negative selective pressure on genes predisposing to CD. They reported a high prevalence of CD in the Middle East region. Wheat has been a major component of the Iranian diet for many centuries and continuous and high level of exposure to wheat proteins may result into some degrees of immune tolerance, leading to milder symptoms that may be misdiagnosed as irritable bowel syndrome in some parts of the country. This may explain our findings and account for the difference with northern parts.

CONCLUSION

We found that CD in our area is not prevalent as the northern parts of the country. High rates of CD in our country may be an overestimation of the disease and we suggest other studies to include serology, HLA typing, histology, and also follow-up studies in healthy people and high-risk groups to determine the true prevalence of CD in Iran.

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