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Alimentary Tract

Prevalence of celiac disease among first-degree relatives of Indian celiac disease patients

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ABSTRACT

Background: Celiac disease, once thought to be uncommon in Asia, is now recognized in Asian nations as well. We investigated the prevalence of celiac disease in first-degree relatives of celiac disease patients followed in our centre.
Methods: First-degree relatives were screened prospectively for celiac disease using questionnaire-based interview and anti-tissue transglutaminase antibody. Serology positive first-degree relatives underwent duodenal biopsies. Diagnosis of celiac disease was made based on positive serology and villous abnormality Marsh grade 2 or higher. Human leucocyte antigen DQ2/-DQ8 was also assessed in 127 first-degree relatives.
Results: 434 first-degree relatives of 176 celiac disease patients were prospectively recruited; 282 were symptomatic (64.9%), 58 were positive for serology (13.3%). Seroprevalence was higher in female than in males (19% vs 8.5%; $p = 0.001$) and highest in siblings (16.9%) than parents (13.6%) and children (5.9%) of celiac patients ($p = 0.055$); 87.4% first-degree relatives were human leucocyte antigen-DQ2/-DQ8 positive. Overall prevalence of celiac disease was 10.9% amongst first-degree relatives.
Conclusions: The prevalence of celiac disease in first-degree relatives of celiac disease patients was 10.9% in our cohort, and 87% had human leucocyte antigen-DQ2 or -DQ8 haplotype. All first-degree relatives of celiac disease patients should be screen for celiac disease even if asymptomatic or with atypical manifestations.

1. Introduction

Celiac disease (CD) is an immune-mediated enteropathy triggered by ingestion of gluten present in wheat and related prolamines present in rye and barley in genetically susceptible individuals carrying HLA-DQ2 and/or HLA-DQ8 haplotype [1]. Once thought to be uncommon, CD affects approximately 1% of the world's population [2–4]. Even in Asian countries, where CD was thought to be a rare disease, recent studies – including one from our centre – suggest a prevalence of 1 in 330 to 1 in 96 in the general population in Northern India [5,6]. CD is now being observed in other Asian countries including China, Malaysia and Pakistan [7–10].

Since CD is a genetic disease, first-degree relatives (FDRs) of patients with CD are at higher risk of developing CD due to close genetic repertoire, which leads to higher genetic susceptibility [4]. Advent of celiac-specific antibodies (a reflection of adaptive immune response to gluten peptide) has revolutionized the case detection rate and has led to recognition of CD as a public health problem world over [11]. With the help of celiac-specific serologic tests, it is now possible to screen and detect CD not only the clinically apparent patients but also those who still have not developed any symptoms. With increasing use of screening and diagnostic tests along with increase in the awareness, CD has become one of the most common genetic disease.

Because of the genetic susceptibility, both FDRs and second-degree relatives are at a higher risk of developing CD than the general population. The prevalence of CD in FDRs vary widely from 5% to 38% [12–15]. The three studies from Asia, all the them from

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the Northern part of India have also reported a prevalence of CD in FDRs to vary from 8.2% to 22% [16–18]. The risk of having CD in FDRs varies with the individual relationship with the index patient with CD. Several studies have suggested that siblings are at a higher risk of developing CD in comparison to parents and children [19,20,13]. The spectrum of symptoms in FDRs varies and a large number of them remain either asymptomatic and or mildly symptomatic. Only a proportion of them have full-blown symptoms.

Since CD is now emerging in Asian countries, and there are two other studies from India with much smaller sample size in both the studies and inclusion of only siblings in one of the study [16,17]. Therefore, we conducted the present study to find out the prevalence of CD in the FDRs of patients with CD.

2. Methods

2.1. Subjects

This prospective study was conducted at All India Institute of Medical Sciences, New Delhi, between May 2009 and September 2014. From the initial cohort of 540 patients with CD followed at our Celiac Disease Clinic, all FDRs were invited to be screened for CD. Four hundred and thirty four FDRs (54% of total live FDRs) of 176 index patients agreed to participate and were enrolled.

This study was approved by the Ethics Committee of our Institution and written informed consent was obtained from each participant and parents or guardians for subjects aged less than 18 years. All FDRs were administered a questionnaire and they underwent a complete history and clinical evaluation.

Five-millilitres of blood was collected and separated in a plain tube (2 ml) and in EDTA (3 ml). Plain tube sample was kept at room temperature for approximately an hour and then centrifuged at $2500 \times g$ for 10 min. Supernatant was separated and was kept in a microcentrifuge tube at 20 °C until analysis. DNA was isolated from the blood collected in the EDTA tubes.

2.2. IgA anti-tissue transglutaminase antibody (IgA anti-tTG Ab)

FDRs were screened for CD using commercially available IgA anti-tTG Ab ELISA kits procured from AESKU Diagnostik, Wendelsheim, Germany (cut-off: 18 IU/ml). FDRs with a positive ant-tTG Ab were invited to undergo upper gastrointestinal endoscopy and duodenal biopsies.

2.3. Endoscopy and mucosal biopsy examination

Upper gastrointestinal endoscopy was performed and at least four to six biopsies were taken from the second or third part of the duodenum. A biopsy fragment was considered oriented when at least 3 crypts were oriented perpendicularly on the underlying muscularis mucosae. Biopsies were analyzed for mucosal changes by a histo-pathologist with special expertise in gastrointestinal pathology blinded to the clinical or serological results. The Modified Marsh grading system was used for grading mucosal changes: Grade 0, normal histology [a crypt to villous ratio of 1:3 was taken as normal]; Grade 1:increase of intraepithelial lymphocytes (IEL) >40/100 enterocytes (IELs were identified as dark round cells with high nucleus to cytoplasmic ratio, in comparison to the perpendicularly oriented cigar shaped vesicular nuclei of the mucosal epithelial cells); Grade 2: increased IELs along with crypt hyperplasia; Grade 3: increased IELs along with crypt hyperplasia and variable degrees of villous atrophy. A further semi-quantitative sub typing of the villous atrophy was performed as follows: grade 3a/mild villous atrophy [duodenal biopsies with a crypt to villous ration of >1:3 but <1]; grade 3b/moderate villous atrophy, C:V ratio of 1:1; grade 3c/severe villous atrophy, C:V ratio of >1.

The diagnosis of CD was made on the basis of the modified European Society of Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) criteria i.e., clinical features, presence of villous atrophy (villous abnormalities of modified Marsh grade ≥ 2) and unequivocal response to gluten-free diet [21].

2.4. HLA genotyping

The genotyping for HLA-DQ2 and -DQ8 was performed using reverse sequence specific oligonucleotides method (One Lambda, Inc., Thermo Fisher Scientific, USA). Readings were taken through Luminex X-Map technology (Luminex, Life Technologies, Inc).

2.5. Statistical analysis

Quantitative variables were summarized as mean ± standard deviation. Qualitative were summarized as frequency (%). Qualitative variables were compared between those with a positive and negative anti-tTG Ab using Chi-square test. Quantitative variables were compared using student's *t* test or Wilcoxon's Ranksum test as appropriate.

3. Results

Overall, 434 FDRs of 176 CD patients (54% of all living FDRs) were included in this study; 234 (53.9%) were males, mean age was 29.8 ± 15.9 years; 326 were over 18 years of age (75.1%). Relationships with patients were 159 siblings (36.6%), 191 parents (44%), and 84 children (19.3%). Four FDRs had previously been diagnosed with CD and were already following gluten-free diet. FDRs who agreed to participate were younger than those who did not consent (29.71 ± 15.95 vs 37.38 ± 16.7 years). However, there was no difference in gender distribution.

3.1. Symptoms

Two hundred and eighty two FDRs (64.9%) had one or more symptoms; 140 had predominantly gastrointestinal symptoms (32.2%) and 253 had a combination of both gastrointestinal and extra intestinal symptoms (58.2%; Table 1); 152 were completely asymptomatic (35%). Hypothyroidism was present in 4.

3.2. Seroprevalence of CD

Overall, 58 FDRs belonging to 44 index patient families tested positive for anti-tTG Ab (13.3%). This meant approximately 25% (one in 4) of families had at least one anti-tTG Ab positive FDR. Five families of index CD patients had 2 or more anti-tTG Ab positive FDRs.

CD seroprevalence was higher in female than in male FDRs (19% vs 8.5%; $p = 0.001$). The seroprevalence was 16.9% in siblings vs 13.6% in parents and 5.9% in children ($p = 0.055$) of CD patients.

Amongst anti-tTG Ab positive FDRs, high titre (>5 times and 3–4 times above the cut-off value for the positive test) of anti-tTG Ab was more common in females than males (Table 2).

3.3. Association between symptoms and anti-tTG Ab status

FDRs with positive anti-tTG Ab test were more frequently symptomatic ($p = 0.044$), and extra-intestinal symptoms were significantly more common in anti-tTG Ab-positive than negative anti-tTG Ab FDRs ($p = 0.004$). Amongst gastro-intestinal symptoms, vomiting was more common in anti-tTG Ab positive FDRs than those with a negative anti-tTG Ab test ($p = 0.044$). Amongst non-gastrointestinal symptoms, failure to gain weight was significantly