

HOW COMMON IS CELIAC DISEASE IN EASTERN SAUDI ARABIA?

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Background: In contrast to its prevalence in Europe, celiac disease (CD) is considered rare in non-Caucasian populations. We aimed to estimate the prevalence of CD in clinically suspicious celiac disease patients and in patients with disorders known to be associated with CD, such as autoimmune diseases, using serological assay for IgA-endomysial antibodies (EMA) on inexpensive human tissue substrate.

Patients and Methods: IgA-endomysial and IgA-reticulin antibodies (ARA) were evaluated by indirect immunofluorescence (IIF) study using human umbilical cord (HUC) and rat tissues, respectively, in the following groups: group 1, 145 patients with clinical suspicion of CD; group 2, 80 with autoimmune diseases; group 3, 20 patients with inflammatory bowel disease (IBD); and group 4, 100 healthy blood donors.

Results: Of the 145 patients with suspected CD (group 1), 11 were EMA positive with or without ARA, giving a serological prevalence of 7.6%. The histological findings of intestinal biopsy were confirmed in six of them, indicating a CD prevalence of 4%. In group 2, two EMA-positive (2.5%) with or without ARA cases were found. Both were from the 18 patients who had autoimmune thyroid disease, indicating an 11% prevalence of EMA in autoimmune thyroid disease. No positive EMA was detected in the 3rd and 4th groups, indicating 100% specificity.

Conclusion: Our findings showed high prevalence of CD in a reference laboratory setting. This highlights the importance of keeping CD in mind and of promptly investigating suspected individuals. There is also a high prevalence of CD among patients with autoimmune thyroid diseases, and further studies are needed to elucidate the significance of this association. Test for endomysial antibodies using human umbilical cord is an inexpensive, easily available and highly specific tool for identifying patients to undergo biopsy and to screen at-risk groups of patients.

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Celiac disease (CD) is an inflammatory disorder of the upper small intestines, most probably caused by an abnormal immune reaction to wheat gliadin. The disease is characterized by intestinal villous atrophy and crypt hyperplasia. A range of symptoms and signs may be associated with CD, depending on the degree of intestinal involvement. Thus, the disease can be overt, with the classic features of diarrhea, abdominal distension, generalized malnutrition and failure to thrive, or subclinical, with isolated nutrient deficiencies such as anemia, aphthous ulcer, bone pain, etc., without gastrointestinal symptoms, particularly at a later age. Incidence of gastrointestinal carcinoma or lymphoma increases among patients with untreated CD.¹

Until about a decade ago, CD was considered uncommon even in Europe, with a prevalence rate of 1/1000 or lower.² The disease is thought to be rare to non-existent among blacks and Asians.^{1,2} However, several recent population studies have shown a much higher prevalence, and it is now estimated that CD may affect 1/200 or even greater.² The iceberg is a common model to explain the epidemiology of CD.² Accordingly, only a minority have the clinically recognized disease while the majority of patients have silent CD, which remains undiagnosed because the condition has no symptoms or has symptoms which are unrelated to intestinal manifestation.

The discovery that CD is a prevalent disorder can be attributed to the judicious use of serological screening tests which measures anti-endomysial (EMA), anti-gliadin (AGA) and anti-reticulin antibodies (ARA). Among the three serological markers, IgA-endomysial is the most reliable marker when sensitivity, specificity and positive-predictive values are taken together,^{2,5} while antigliadin is the least sensitive and specific.^{4,5} Nevertheless, we found EMA the least frequently requested marker when our clinicians suspect CD, while antigliadin was the most frequently

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FIGURE 1. Immunofluorescent staining pattern of EMA in cryostat section of human umbilical cord.

TABLE 1. Characteristics of the four groups and their autoantibody profiles.

	Group 1	Group 2	Group 3	Group 4
Number	145	80	20	100
Clinical characteristics	Suspected celiac disease	Autoimmune diseases	Inflammatory bowel disease	Healthy donors
IgA-ARA	9	1	0	1
IgA-EMA	11	2*	0	0
Prevalence of EMA	7.6%	2.5% (11%)*	0	0

*Both were from the 18 patients who had autoimmune thyroid disease (AITD), this makes prevalence of EMA 11% among AITD subgroup.

TABLE 2. Characteristics of the 11 patients with suspected Celiac disease.

Age/Sex	Symptoms	EMA	ARA	Results of intestinal biopsies
36/M	Abdominal pain	+	+	Compatible with CD
10/F	Abdominal pain	+	+	Compatible with CD
1.4/F	Chronic diarrhea	+	+	Not done
1.25/M	Chronic diarrhea	+	+	Not done
36/F	Abdominal discomfort, anemia	+	-	Compatible with CD
0.9/F	Chronic diarrhea	+	+	Not available
2/M	Diarrhea, failure to thrive	+	+	Not available
1.8/M	Diarrhea, failure to thrive	+	+	Compatible with CD
5/M	Short stature	+	-	Compatible with CD
1.5/M	Failure to thrive	+	+	Compatible with CD

requested. As a test, EMA uses indirect immunofluorescence technique (IIF) on monkey esophagus sections, however, human umbilical cord (HUC) has recently been found to be a suitable alternative substrate.^{6,7}

The scarcity of data on the prevalence of CD among the Arab population—there is only one available to date⁸—in addition to a recent report by Catassi et al.⁹ who showed a prevalence of 5.6% in the Western Sahara, prompted us to investigate this problem in the Saudi population. Thus our aims are: 1) to estimate the prevalence of CD among patients submitted to a reference laboratory for symptoms suggestive of CD and among patients with diseases known to be associated with CD, such as autoimmune disorders; 2) to increase the awareness of pediatricians and clinicians about the value of EMA as an excellent diagnostic tool in the diagnosis of CD; and 3) to encourage the use of an easily available inexpensive substrate (human umbilical cord) for the identification of EMA, particularly in screening at-risk groups of children.

Patients and Methods

Sera were collected for evaluation of anti-reticulin (ARA) and anti-endomysial antibodies (EMA) by IIF test from four groups: group 1 consisted of 145 consecutive

patients referred for suspected celiac disease between January 1995 and August 2000. These patients were referred from six district hospitals to the Department of Immunology at the Regional Laboratory and Blood Bank, Dammam, which is the sole provider of immunological diagnostic services in Eastern Saudi Arabia. One or more of the following symptoms were the cause for referral; chronic diarrhea, failure to thrive, chronic abdominal discomfort short stature or symptoms suggestive of malabsorption syndrome. There were 95 children (66%), with a mean age of 4 years (range 6 month - 15 years) and 50 adults (34%), with a mean age of 40 years (range 17-70 years). Sixty-four were females representing 44% of the total and 81 were males (56%). Group 2 consisted of 80 consecutive patients, referred in the previous two years of the study period for evaluation of autoimmune diseases. This group was included because of the reported higher than expected prevalence of CD in patients with autoimmune disorders.² The autoimmune diseases consisted of the following conditions: 29 insulin-dependent diabetes mellitus (IDDM), 18 autoimmune thyroid disease, 1 case of alopecia, 2 vitiligo, 22 connective tissue disease (CTD) and 8 multiple autoimmune diseases, with the latter including two or more of the following conditions; autoimmune hepatitis, autoimmune gastritis and autoimmune thyroid disease. Investigations of other autoimmune disorders were not available.

To determine the specificity of endomysial antibodies on the new substrate (human umbilical cord) we investigated this marker in two different groups: group 3 consisted of 20 patients with inflammatory bowel disease (disease control); and group 4 consisted of 100 blood donors (normal control). The inclusion of the fourth group also served as a step in an ongoing screening program for silent cases.

Indirect Immunofluorescence (IIF) Study

This was used for the detection of autoantibody profile, including antinuclear (ANA), anti-smooth muscle (ASMA), anti-mitochondrial (AMA) and anti-gastric parietal cell antibodies (GPC) and anti-Islet cell antibody (ICA) using standard IF techniques and ANA/Hep-2 cell culture (INCSTAR Corp., USA), unfixed cryostat (4 µm) sections of rat tissues and commercial slides of monkey pancreas (Inova Diagnostic Inc., USA). IgA-ARA and IgA-EMA were detected on commercial slides of rat kidney and monkey esophagus, respectively, according to the instruction of the kit used. ELISA for detection of AGA was unavailable. The supply of monkey esophagus substrate was not consistent throughout the year, thus determination of EMA was carried out on 5 µm thin cryostat sections of HUC. Preparation of the sections was done as described by Lan Dinser et al.,⁶ with minor modifications as follows: the umbilical cord was cut, lightly pressed and placed on a pre-frozen microtome chuck. OCT (Diachem Int. Ltd., UK) was applied and the chuck was

mounted on a freezing microtome (cryostat). The section was then either kept there or cut immediately, mounted on multispot glass slides and air dried for 30 min. The slides were then either stored in an air tight box up to 3 months at -20°C or used immediately as follows: the sections were fixed for 5-10 minutes in acetone at -20°C , washed in phosphate buffered saline (PBS) 0.05M pH 7.4 and incubated with 1% bovine serum albumin (Sigma, Italy) for 10 minutes. Staining for IIF was performed as described elsewhere.¹⁰ Briefly, patient's sera were applied at 1:5 and 1:10 dilutions with PBS incubated in moist box for 30 minutes at room temperature. After washing with PBS, the sections were incubated in a dark room with fluorescence isothiocyanate (FITC) conjugated rabbit anti-human IgA (Inctar molar fluorochrome/protein ratio of 2-4, protein concentration >5 mg/mL) at 1:30 dilution in PBS for another 30 minutes, washed in PBS, mounted in aqueous mounting medium (Immco Diagnostics, New York) and examined using Nikon epi-illumination microscope (Nikon, Tokyo, Japan). Positive and negative controls were used for each batch. Only after the results of EMA assay on HUC were in accordance with those obtained using conventional assay on monkey esophagus sections on five consecutive positive and five consecutive negative sera were patient sera assayed on HUC solely.

Antimicrosomal and anti-thyroglobulin antibodies for autoimmune thyroid diseases were determined using hemagglutination kit (Abbott Murex Biotech, UK). Sera of the patients that were not tested immediately were kept at -70°C . All the sera were collected while the patients were on normal diet.

The hospital charts of all patients in whom a diagnosis of autoimmune disease or CTD had been made were reviewed thoroughly to verify whether the recognized diagnostic criteria for each disorder had been fulfilled. The histological reports of intestinal biopsies were evaluated for those who had positive assays for EMA and ARA.

Results

Eleven patients (5 female and 6 males) comprising nine children with a mean age 2.8 years with suspected CD (group 1) had both ARA and EMA or only EMA antibodies (9 positive for both, 2 had EMA only), giving an overall prevalence of 7.6%. Their sera showed the characteristic reticular fluorescence pattern of EMA along the peritubular muscle layers of vessels on umbilical cord (Figure 1) or around smooth muscle fibers in the muscularis mucosa on monkey esophagus. On reviewing the medical records of these patients, the diagnosis of CD was confirmed in six, by showing the typical histological changes of intestinal biopsy seen in CD. The medical records of two patients could not be traced, while in the remaining three patients, biopsies were not done, (all were less than 2 years). In study group 2, two patients were positive for EMA (plus ARA in one case) giving a

prevalence of 2.5%. Both patients had autoimmune thyroid disease. In groups 3 and 4, no positive results for EMA marker could be detected, which gives 100% specificity for EMA. Only one healthy blood donor demonstrated weak positive ARA. Table 1 summarizes the characteristics and the auto-antibody findings of the study groups, and Table 2 shows the characteristics of the 11 suspected CD patients who had positive EMA.

Discussion

Both the heterogenous manifestation and the increasingly recognized range of associated diseases and extraintestinal manifestations that may overshadow gastrointestinal symptoms,¹¹ make diagnosis of CD difficult. Furthermore, the characteristic histological findings are not specific,¹ and may be absent at the early stages of the disease, leading to many cases being undiagnosed. The European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) has recommended the inclusion of various serological tests to reduce the number of intestinal biopsies to make a diagnosis.¹² These include tests for anti-gliadin (AGA), anti-reticulin (ARA) and anti-endomysial (EMA) antibodies, which are generally noninvasive and easy to perform.

Although small intestinal biopsy remains the gold standard for diagnosis, there is no doubt that the modern serological tests of EMA, AGA and ARA autoantibodies have become more reliable for better targeting of patients for biopsy and for monitoring dietary treatment. In addition to the superiority of EMA in terms of diagnostic performance,^{4,5} the fluorescence of EMA is clearer and easier to read than ARA, thus it has been suggested as the test of choice for screening within a routine laboratory.³ The only disadvantage with EMA is the use of expensive and ethically questionable monkey esophagus substrate. A major advance in this area is the identification of tissue transglutaminase (t-TG) as the target antigen for EMA, which can be detected by ELISA.² Another advance, which is far less costly, is the HUC used in this study as an alternative substrate for the detection of EMA by IIF. In our experience, the method based on the use of umbilical cord is simple, inexpensive and the actual technique is routine in most immunology laboratories.

Our study showed that the attitude of clinicians in requesting AGA in preference to EMA led to the misdiagnosis of many cases. This was obvious when we looked at the distribution of new cases per year (data not included). We found that 87% of the cases were identified over the last two years of the study when we implemented a policy of performing assay for EMA for all referred cases of suspected celiac disease, regardless of the requested test.

Regional differences have been reported in the prevalence of CD in Western countries.¹³ Although case documentation had been reported from tropical and some Arab countries,^{14,15} little information is known about the

disease prevalence in Arabs. We identified 11 patients with suspected CD who had positive EMA, giving a prevalence of 11/145 (7.6%). This finding supports the high frequency in Arab population which was speculated by Catassi et al. to be due to selective advantage afforded by celiac enteropathy to affected individuals in the form of protection against intestinal infections/infestations.⁹ Whether this increased prevalence in Arabs is real or reflect selective bias can only be solved by community-based surveys. Although, histological findings were confirmed in only six patients, which lowered the prevalence to 4%, we believe the prevalence is higher. Taking into account the 100% positive predictive value of EMA in the typical clinical setting, it is possible to use EMA as a valid alternative to duodenal biopsy when the latter is not feasible.¹⁶ Furthermore, there will be always some patients who are unwilling to undergo small intestinal biopsy, and it is also possible to get negative biopsy due to patchiness of the lesions in some patients.¹⁷ Thus, we believe calculation of the prevalence based only on confirmed cases will underestimate the true prevalence. Practically speaking, patients in whom confirmation by intestinal biopsy could not be obtained are considered probable CD cases, and advised to follow gluten-free diet. Even though a prevalence of 4% is considered high compared to similar hospital-based studies such as those reported from USA (3%)¹⁸ and Europe¹ (between 1:300-1:1000), it is comparable to prevalence rates reported from tropical countries.¹⁴

For unexplained reasons, Italian patients have the highest prevalence rates (47%).⁵ Endomysial antibodies have never been found in healthy controls, and have so far been negative in patients with Crohn's or ulcerative colitis.³ Therefore, our results of negative EMA in groups 3 and 4 confirm this high specificity and is in concordance with most laboratories.¹⁹

Population screening suggests high rates of undiagnosed celiac diseases in certain Western communities.²⁰ This was not the case among our healthy donors, which showed absence of silent CD, however, the numbers were too small to draw a firm conclusion. The striking association between HLA class II molecules, DQ2 and DQ8 and celiac disease^{1,2} implicates a role for immune response in disease pathogenesis. This has been supported by the frequent association between CD and some autoimmune disorders such as dermatitis herpetiformis (DH) and IDDM.¹

We could not identify a single case of DH during the study period, suggesting the rarity of this condition in our population. We found two cases of possible CD in the autoimmune disease group (group 2), which gives a prevalence of 2.5%, however, since both had autoimmune thyroid disease, a prevalence of 11% among the 18 patients who had autoimmune thyroid disease can be speculated. This prevalence is much higher than the reported 3.4% in similar conditions.²¹ The significance of these markers remains to be determined, particularly with the reported

transient autoantibodies in some patients.²² However, because there is evidence that long-standing CD, even if silent, predisposes to autoimmune disease, a screening program for EMA in such cases seems justified.²³ IgA deficiency has also been reported to be associated with CD,^{1,2} although we did not look for the prevalence of CD among IgA-deficient patients despite our report of the latter condition in our population,²⁴ however, this is part of an ongoing study to look for this association. In patients with IgA deficiency, assays for antigliadin (AGA) of IgG isotype can be helpful, thus enabling almost all patients to be identified, especially those who would possibly have been missed when IgA-EMA was used.

In conclusion, our study showed high prevalence of CD in Saudi patients. It also showed a high prevalence of serological markers of CD among patients with autoimmune thyroid disease. A similar high prevalence could be expected in other autoimmune diseases and possibly in the general population. Further screening of the population is needed to estimate the true prevalence. Finally, our local laboratories should consider the obvious cost effectiveness of HUC as an alternative substrate for the detection of EMA; particularly in screening latent cases in "at risk" children. This program is highly recommended as CD is pre-malignant and has long-term complications on one hand, and excellent prognosis following gluten-free diet on the other.

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