

Development, validation and registration of the HeberFast Line[®] anti-transglutaminase system. Contribution to the diagnosis of Celiac Disease in Cuba

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Introduction

Celiac disease (CD) is one of the most common food intolerance-mediated enteropathies in man. The conclusive diagnosis of CD is carried out by intestinal biopsy, which detects the histological changes characteristic of this disease. However, less invasive serological screening methods, such as those detecting anti-gliadin (AGA), anti-endomysium (EMA) or anti-tissular transglutaminase (ATA) antibodies, have gained attention due to their lower cost [1].

Although commonly regarded as a European disease rarely seen in the Americas, it is currently recognized that the prevalence of CD is as high in USA and Canada as in the European countries [2, 3]. Other studies carried out in Brazil and Argentina have evidenced that CD is also frequent in Latin American countries [4, 5].

CD has been associated with a higher incidence of intestinal lymphoma when undiagnosed or not treated early. Since the treatment consists of a gluten-free diet (GFD) for the entire life of the patient, an early and precise diagnosis by intestinal biopsy is considered necessary [6, 7]. In order to reduce the number of biopsies needed for an accurate diagnosis, the *European Society for Paediatric Gastroenterology, Hepatology and Nutrition* (ESPGHAN) recommends the use of serological tests to evaluate the levels of AGA, EMA or ATA in the sera of these patients.

In Cuba, CD has been studied since the early 80's based on clinical evidence and intestinal biopsies [8-10]. The diagnosis is currently based on clinical symptoms and AGA tests, confirming the results by biopsy if the former are positive. However, the low specificity of the AGA assay leads to unnecessarily high numbers of biopsies [1, 7]. Recently, a visual detection immunoassay was developed for the evaluation of ATA [6], driven by the need for the implementation of a massive screening of CD among risk groups and in the general population [1, 6, 7, 10-14].

The HeberFast Line[®] anti-transglutaminase system

The Heber Fast Line[®] Anti-transglutaminase test (Figure 1) is a fast, simple and single-step assay to detect IgG and IgA antibodies in the serum, plasma or human blood that does not require prior processing of the samples and can be used under field conditions, nee-

ding only 15-20 min for its completion. It consists of a compact kit especially designed for manual operation and the visual reading of results. The kit is recommended for the screening of patients with symptoms suggestive of CD, asymptomatic individuals with a familial history of CD, patients affected by diseases frequently associated to CD (insulin-dependent Diabetes mellitus, Down's syndrome, and selective deficiency of IgA), for monitoring the efficacy of GFD in

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A. Kit



B. Components (lancets, capillaries, cassettes and booklet)



C. Expected results

Figure 1. The Heberfast Line[®] Anti-transglutaminase system.

CD patients, and for the implementation of CD prevalence studies in the population. This test was awarded the Cuban National Health Award in 2007, under the category of technological innovation.

Clinical sensitivity and specificity

Serum samples from 50 untreated celiac patients, diagnosed according to the ESPGHAN criteria, were screened to evaluate the outcome of the reference test. The result was positive for all of them, for a sensitivity of 100% (95% CI, 92.9-100%) [6].

The same samples were evaluated by an ELISA commercial test (Celikey Pharmacia & Upjohn, Freiburg, Germany) for IgA antibodies against transglutaminase and IgA antibodies against monkey endomysium (Bio System, Barcelona, Spain). Forty-seven samples were positive by ELISA, for a sensitivity of 94% (95% CI, 88.2%-98.4%), and 48 were positive by indirect immunofluorescence, for a sensitivity of 96% (95% CI, 89.1%-99.0%). Samples from 40 non-celiac patients with intestinal disorders were also tested to evaluate the specificity of the assay. In this case, all samples were negative by the three systems with 100% specificity (91.2%-100%). The sensitivity and specificity of the three systems (immunochromatographic assay, ELISA and indirect immunofluorescence) were calculated based on the results of jejunal biopsies as the gold standard for CD [6].

Celiac disease prevalence studies in risk groups and healthy population

Patients with symptoms suggestive of CD

Only 88 patients (39 males and 49 females) out of 637 with classical CD symptomatology and AGA-positive for who would comply with the criteria for jejunal biopsy were positive for ATA, accounting for a seroprevalence of 13.81%. They averaged 12 ± 10.91 years old. Fifty-seven patients were subjected to jejunal biopsies, which yielded a histological pattern compatible with CD in 56 of them; no histological evidence of CD could be detected in the remaining patients after two biopsies. These studies showed a biopsy-proven prevalence of 8.95% in this group of patients [1]. Based on this data, we present in figures 2 and 3, respectively, the current algorithm and that proposed for this group of patients, once the ATA determination is introduced into the routine clinical practice.

Giardiasis patients

Thirty-seven out of 40 giardiasis patients showed a normal structure of the intestinal microvilli. The other three patients presented subtotal microvilli atrophy and lymphocytic intraepithelial infiltration, compatible with a diagnosis of CD [7]. The severity of the lesions was very similar in these three patients and did not allow for further differentiation by histological observation alone; nevertheless, only two of the patients were positive for ATA and therefore classified as suspected CD patients, who were assigned to GFD therapy and additionally treated for giardiasis. The third patient, showing subtotal mucosal atrophy but negative for ATA, was only treated with secnidazole. A clinical improvement was observed for both suspected CD patients, together with a histological

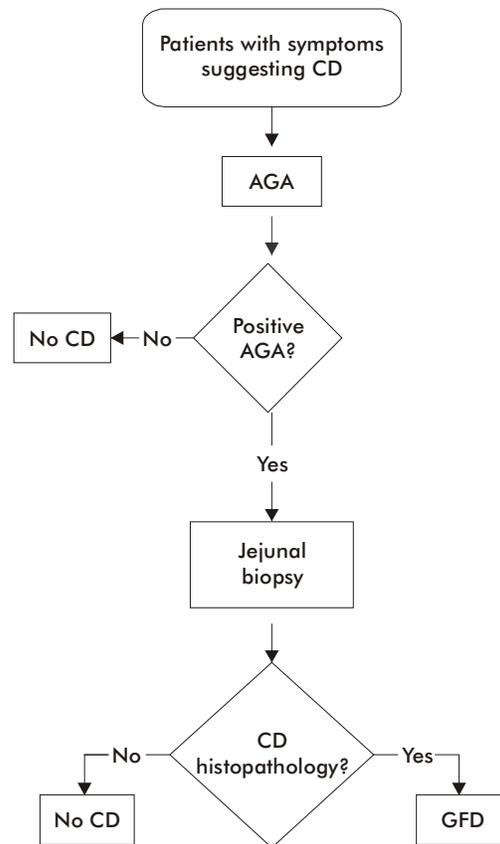


Figure 2. Current diagnosis algorithm for celiac disease (CD) in Cuba. AGA-anti-gliadin antibodies. GFD-Gluten-free diet.

recovery established in a second intestinal biopsy and their conversion to an AGA seronegative status, thus confirming the diagnosis according to the ESPGHAN revised criteria. A histological recovery was also observed for the third patient (secnidazole treatment alone) after a second intestinal biopsy. Additionally, his ATA status remained negative [7].

Diabetes mellitus type 1 patients

Fourteen (9 females and 5 males) out of 208 type 1 diabetes mellitus patients were positive for ATA, for a 6.73% seroprevalence. Only two of them (14.28%) showed clinical symptoms associated to CD (abdominal pain, diarrhea and anorexia). The confirmation of CD was performed by jejunal biopsies in the 6 patients who consented with the assay, yielding morphological changes consistent with CD for all of them [14]. Five of the patients presented partial moderated atrophy of the intestinal microvilli with an increased number of intraepithelial lymphocytes. The other patient showed subtotal microvilli atrophy. In general, the prevalence of CD diagnosed by intestinal biopsy in this risk group is 2.88% (6/208). The average age in the group of diabetic patients positive for ATA and confirmed as having CD was 9 ± 1.79 years, with statistically significant differences when compared to the group of ATA negative diabetic patients ($p < 0.0044$). The mean age at CD diagnosis for this group of patients was 11 ± 4.56 years old. The average interval

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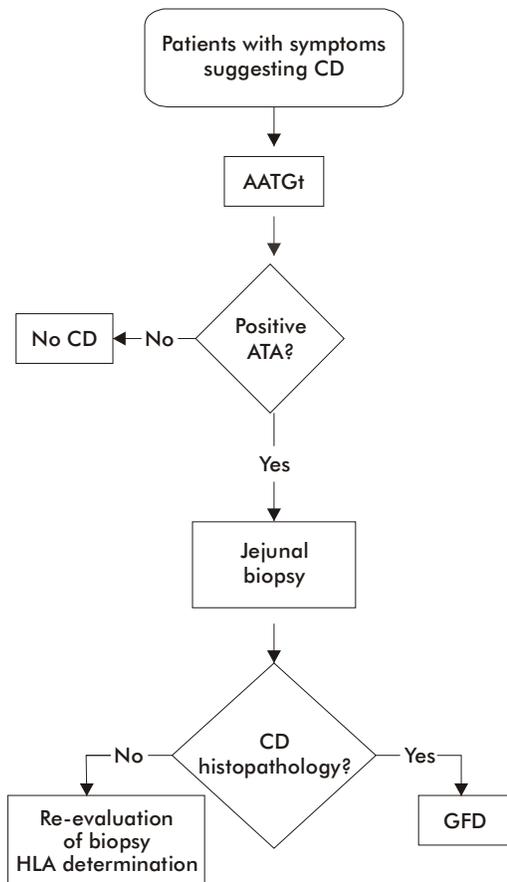


Figure 3. Algorithm proposed for the diagnosis of celiac disease (CD). ATA - anti-tissular transglutaminase antibodies. GFD- Gluten-free diet. HLA- human leukocyte antigens.

between the diabetes diagnosis and that of CD was of 2 ± 4.00 years. In this group of diabetic patients who were positive for CD, four were diagnosed at the onset of diabetes, and the other two, at 2 and 10 years after the onset of diabetes, respectively. None of these two patients showed symptoms indicating CD. It is worth noting that none of the patients studied had a first-grade familial history of CD [14].

Studies of the prevalence of CD in healthy pediatric and adult populations

Two groups of 500 children and 200 adults were screened for ATA. Patients with positive results were remitted to Gastroenterology services to confirm the diagnosis of CD by jejunal biopsy, and also to study the alleles of their major histocompatibility class II system (HLA II) genes associated to CD.

The seroprevalence of ATA in the main groups studied is shown in table 1.

HLA DQA1*0501 and DQB1*02 alleles in Cuban celiac patients and their first degree relatives

Susceptibility to CD is highly associated to class II HLA-DQ2 and HLA DQ8 genes [11]. For the first time in Cuba, the HLA DQA1*0501 and DQB1*02 (DQ2) alleles were characterized in a group of 136

Table 1. Seroprevalence of ATA. Pos-positive; Neg-negative; Ref-references

Groups	Pos	Neg	Total	Seroprevalence (%)	Ref.
Diabetes mellitus type 1	14	194	208	6.7 (2.8)*	[14]
Diabetes mellitus type 2	3	155	158	1.9	[12]
Down's Syndrome	6	257	263	2.0	[1, 13]
Giardiasis	2	38	40	5.0*	[1, 7]
Hypertransaminasemia	1	114	115	0.87	[1]
Patients with symptoms suggesting CD	88	549	637	13.8 (8.95)*	[1]

*Prevalence as confirmed by jejunal biopsy.

individuals from several provinces. Twenty-two of them were celiac patients diagnosed by jejunal biopsy, with 54 first-degree relatives and 60 healthy subjects as controls. All individuals were assessed for serum ATA, and were positive for ATA in 100%, 19% and 0% of the cases, respectively.

It was established in the genetic study that 86.3% of the subjects were positive for the DQA1*0501 allele, 90.2% for the DQB1*02 and 86.3% for both alleles. The frequency distribution of the alleles in the group of relatives was of 70% (DQA1*0501), 90% (DQB1*02) and 70% (both alleles), and of 56.6%, 45%, and 20% in the control group of healthy subjects, respectively. It is noteworthy that 7 out of the 10 first-degree relatives that were positive for ATA were also positive for both alleles of the HLA DQ2, and of these 7, 5 were positive and 2 were negative in the jejunal biopsies [11].

Conclusions

The HeberFast Line® anti-transglutaminase system was the first CD diagnosis system in the world that can detect IgA and IgG antibodies in a single assay. This could reduce in more than 30% the number of jejunal biopsy studies carried out at the Gastroenterology Services in Cuba, with significant savings in resources and costs of care. This test also avoids the need for biopsies in patients with an uncertain diagnosis. The specificity of the serological assay for ATA means that the probability of a negative result after a biopsy is low.

The prevalence studies carried out in some risk groups, and in healthy children and adults, showed that CD is more frequent than previously considered in Cuba. These observations reinforce the need for conducting extended prevalence studies for this disease in our country. The study of CD-associated genotypes, performed for the first time in Cuba, showed that the number of patients bearing the HLA DQ2 allele is very similar to that of other populations with a different genetic background. It is frequent to find ATA-positive, first-degree relatives of celiac patients, who suffer from a clinically silent variant of the disease, only evidenced upon jejunal biopsy.

The introduction of the reference test in Cuba would allow the implementation of massive CD screening programs, which would position Cuban Medicine at the leading edge of research on this disease. The system is novel, compared to those existing in the international market for these purposes, e.g., ELISA-like systems which have longer assay times and require independent tests to detect IgA and IgG antibodies. It is easy to handle and adaptable to the conditions of tropical and developing countries with

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scarce resources, requiring neither sophisticated laboratories nor highly trained personnel.

The HeberFast Line® anti-transglutaminase system has a sanitary registration in Cuba and patents granted in Cuba, USA, the European Community, Russia, Argentina and Canada, and also pending patent applications in Venezuela and México.

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