Screening for celiac disease in a healthy Cuban children cohort from Pinar del Río province

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ABSTRACT

Recent studies suggest that celiac disease is common in many developing countries. Taking into account the disease may be underdiagnosed in Cuba, the main objectives of this study were to assess the presence of celiac disease related to antibodies in a cohort of apparently healthy children from Pinar del Río province and to evaluate a new rapid test for detecting celiac disease antibody in blood, serum and plasma samples. A total of 595 apparently healthy children with no record of first degree relatives suffering from celiac disease, were screened for Tissue transglutaminase antibodies by one-step immunochromatographic. The results were compared with commercials ELISA kits. In the study seven subjects (1.18%) were identified as positive by immunochomatographic assay and by Celikey IgG Antibody Assay with a 100% of concordance and only five subjects (0.84%) by Celikey IgA Antibody Assay. The achievement of the intestinal biopsy was offered to all positive individuals. This study demonstrates that one-step immunochromatographic assay is an appropriate tool to detect celiac disease-associated to antibodies and provides further evidence of the prevalence of possible undiagnosed celiac disease among healthy children in Cuba.

Keywords: Celiac disease, anti-tissue transglutaminase antibodies, immunochromatographic, prevalence, biopsy, Cuba

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RESUMEN

Pesquisaje de la enfermedad celiaca en un grupo de niños cubanos sanos de la provincia de Pinar del Río. Estudios recientes sugieren que la enfermedad celiaca es común en muchos países en desarrollo. Debido a que en Cuba existe un subregistro en el diagnóstico de esta enfermedad, el principal objetivo de este estudio fue determinar la presencia de anticuerpos asociados a la enfermedad celiaca en un grupo de niños aparentemente sanos de la provincia de Pinar del Río y además evaluar una nueva prueba de diagnóstico rápido para la detección de anticuerpos anti-transglutaminasa en sangre, suero y plasma. Se estudiaron un total de 595 niños aparentemente sanos que no tenían antecedentes de familiares de primer grado con enfermedad celiaca, a todos se les realizó determinación de anticuerpos anti-transglutaminasa con un ensayo inmunocromatográfico de un solo paso. Los resultados se compararon con sistemas comerciales tipo ELISA. En el estudio 7 individuos (1.18%) fueron identificados como positivos tanto por el ensayo inmunocromatográfico como por el ensayo ELISA Celikeyyyyyy de detección de anticuerpos IgG para un 100% de concordancia y solo 5 sujetos (0.84%) resultaron positivos para el ELISA Celikey de detección de anticuerpos IgA. A todos los individuos que resultaron positivos por cualquiera de los tres ensayos, se les ofreció la realización de la biopsia intestinal. Este estudio demuestra el valor del ensayo inmunocromatográfico de un solo paso como una herramienta útil para la detección de anticuerpos asociados a la enfermedad celiaca y nos proporciona una prueba más de la posible prevalencia de enfermedad celiaca no diagnosticada en niños sanos en Cuba.

Palabras clave: enfermedad celiaca, anticuerpos anti-transglutaminasa, inmunocromatográfico, prevalencia, biopsia, Cuba

Introduction

Celiac disease (CD) is the most common severe food intolerance in the Western world and is due to gluten ingestion in genetically susceptible children and adults. The conclusive diagnosis of CD is carried out by intestinal biopsy, which evidences the histological changes, a characteristic of this disease. However, serological screening methods, such as those detecting anti-tissue transglutaminase antibodies (tTGA), have gained attention being cheaper and less invasive [1, 2].

CD is now considered a public health problem worldwide. CD affects as much as 0.5% to 1.0% of European or European ancestry populations, but most cases remain undiagnosed [2]. Other studies carried out in Brazil (1:681) and Argentina (1:167) brought about that CD is also frequent in Latin American countries [3, 4]. New epidemiological studies have demonstrated that this disorder is also common in many developing countries, the highest CD prevalen-
Celiac disease in healthy Cuban children

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Results

In this study, for the first time in Cuba and to our knowledge in the Caribbean region, we attempted to evaluate the prevalence of CD related antibodies among 595 (280 male/315 female) apparently healthy children, who ranged 3 years old. Only seven children (3 male and 4 female) were positive for tTGA as determined in blood by using the immunochromatographic assay. This result was further confirmed by both serum immunohematography and Celkey Tissue Transglutaminase IgG Antibody Assay, accounting for a 1.18% seroprevalence. Among them, only five children were positive by Celkey Tissue Transglutaminase IgA Antibody Assay (see table). All the rest were consistently negative in all assays. For all tTGA-positive children, a biopsy was indicated but their parents refused its achievement.

Discussion

Several studies have demonstrated that CD is common in many developing countries [2-5]. The prevalence of CD is largely established in many South American countries [3, 4] and some African countries have reported a high CD prevalence [2, 5]. In the Caribbean, CD is underestimated for several reasons, for instance, the belief that this disease does not exist and the scarce diagnostic facilities. In Cuba, CD has been traditionally investigated by clinical and histological studies [7], and more recently by serological methods [1, 8-15]. In previous studies, we reported the prevalence of CD in Cuba among risk groups, such as: diabetes mellitus type 1 (2.8%) [1, 14], Down’s syndrome (2.0%) [1, 13] patient with clinical symptoms (8.95%) [1] also in the “not at risk” healthy adult’s population (0.5%) [15].

In this work, we suspected the diagnosis of possible CD by sequentially determining tTGA using the immunochromatographic HeberFast Line® anti-transglutaminase assay (Heber Biotec SA, Havana, Cuba), and anti-IgA and anti-IgG antibodies with Celkey assays, respectively. Among the 595 subjects studied, seven (1.18%) were suspected of asymptomatic CD. Biopsy histological examination of distal duodenum or small intestine specimens remained the gold standard for a definitive diagnosis [2]. Hence, CD diagnosis in tTGA-positive children should be confirmed by biopsy to establish the real prevalence of this disease.

It has been hypothesized that most children with CD are symptomatic early in life because of the high gluten content of infant diets [16]. However, our findings

Materials and methods

Subjects

We determine the presence of tTGA in 595 children who were 3 years old (born in the 12-month period from April 2003 to March 2004), and who lived in three municipalities of Pinar del Rio province, from January 2007 to March 2007. The study group was comprised of 280 males and 315 females in an age range from 3 years to 3 years, 11 months and 29 days. Blood and serum samples were obtained from all children. The blood samples were tested immediately and sera were stored at -20°C until testing. All the children included in the study were asymptomatic with no history of first degree relatives suffering from CD and their parents agreed previously with the terms of the informed consent.

Anti-tissue transglutaminase antibodies

The anti-tissue transglutaminase whole antibody response was tested in the blood for all subjects included in the study using a fast one-step immunochromatographic assay following the manufacturer instructions (HeberFast Line® anti-transglutaminase, Heber Biotec SA., Havana, Cuba) [9, 10]. Briefly, HeberFast Line® anti-transglutaminase assay nitrocellulose strips placed into a plastic cassette were filled with 100 µL either direct blood obtained by finger puncture or serum or plasma. After 20 min, positive samples were detected as two colored lines on the strips, one in the reactive zone and the other in the control zone. A negative assay should show only a single line in the control zone of the strip. Additionally, IgA and IgG anti-transglutaminase antibodies were determined in serum by using the Celkey IgA and Celkey IgG Antibody Assay (Celkey Pharmacia & Upjohn, Freiburg, Germany). IgA and IgG anti-transglutaminase antibodies were also determined in parallel as a whole antibody response with the one-step immunochromatographic assay.

Table. Clinical characteristics of patients with positive tTGA as determined by using the HeberFast Line and Celkey assays

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>HeberFast Line</th>
<th>Celkey</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>tTGA</td>
<td>IgG</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
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<tr>
<td>2</td>
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<td>6</td>
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<tr>
<td>7</td>
<td>+</td>
<td>+</td>
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</table>

anti - tissue transglutaminase antibodies.


A very simple procedure and starting from the direct samples (blood, serum or plasma) [9, 10]. It has been proven as highly sensitive for CD positive samples as non-treated patients [1, 8-11]. These patients must be confirmed by histological examination of biopsy specimens taken from the distal duodenum or the small intestine for definitive diagnosis [2].

In summary, this study demonstrates the presence of CD markers in a sample of apparently healthy Cuban children, from Pinar del Río Province. Our results highlight the relevance of screening for CD among the general population, also demonstrates the suitability of the HeberFast Line® anti-transglutaminase assay (Heber Biotec S.A., Havana, Cuba) as an appropriate tool for this purpose. The non-invasive nature of this assay supports its application to mass screening for CD, in order to prevent medical complications in asymptomatic children.

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