Seropositivity for celiac disease in children and adolescents with short stature

Soropositividade para doença celíaca em crianças e adolescentes com baixa estatura

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ABSTRACT

Objective: To assess the frequency of positive serological marker for celiac disease in children and adolescents with short stature using the human antibody anti-transglutaminase as a screening test.

Methods: This cross-sectional study was conducted from April to September/2004 with 78 children and adolescents selected by convenience when attending the outpatient clinic of two university hospitals of Recife, Northeast Brazil. Cases were children and adolescents with short stature, defined as height-for-age and sex below the 3rd percentile of the National Center for Health Statistics (NCHS, 2000) growth curve. The human antibody anti-transglutaminase (AATGh) was defined as positive when >20U/mL. For those with a positive result, IgA anti-endomysial antibody was assessed. **Results**: Out of the 78 patients evaluated, 41 (53%) were females. The AATGh was positive in 3/78 (3.8%) patients. The IgA anti-endomysial antibody was positive in one patient, who had the highest AATGh concentration. Taking those with positivity for both tests, the seropositivity was 1.3%.

Conclusions: The presence of serological marker of celiac disease in children and adolescents with low stature of low-income families highlights the need for systematic investigation of celiac disease in these patients.

Key-words: failure to thrive; celiac disease; child; adolescent.

RESUMO

Objetivo: Avaliar a frequência da positividade do marcador sorológico para doença celíaca em crianças e adolescentes com baixa estatura, utilizando-se o anticorpo anti-transglutaminase humana como teste de triagem.

Métodos: Estudo descritivo com amostra obtida por conveniência. Foi realizado no período de abril a setembro de 2004 no Ambulatório Geral de Pediatria do Instituto Materno Infantil Professor Fernando Figueira e no Ambulatório de Crescimento e Desenvolvimento do Hospital das Clínicas. Foram considerados casos as crianças e os adolescentes portadores de baixa estatura, definida como aquela abaixo do percentil 3 para idade e sexo, utilizando como referência o gráfico de altura/idade do *National Center for Health Statistics*, 2000. Foi pesquisado o anticorpo anti-transglutaminase humana (AATGh), considerado positivo se concentração >20U/mL e, nos positivos, o anticorpo antiendomísio (AAE).

Resultados: Foram avaliados 78 pacientes, sendo 41 (53%) do sexo feminino. O AATGh foi positivo em 3/78 (3,8%) dos pacientes. O AAE foi positivo em um paciente, naquele com concentração mais elevada do AATGh. Considerando-se a positividade para os dois testes, a soropositividade foi de 1,3%.

Conclusões: A presença de marcador sorológico para doença celíaca em crianças e adolescentes portadoras de baixa-estatura e pertencentes a famílias de baixa-renda aponta para a necessidade de investigação sistemática da doença celíaca nesses pacientes.

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Palavras-chave: insuficiência de crescimento; doença celíaca; criança; adolescente.

Introduction

Short stature has a variety of different causes and its emergence is dependent on multiple factors: genetic programming, endocrine factors and environmental influences. Environment, in this context, encompasses not only the physical, but also the psychosocial, economic and nutritional environment[1,2]. This is a complex phenomenon which, in the majority of cases, is the result of multiple causative mechanisms.

The causes of short stature are described as primary when there is an abnormality in the potential for bone growth, as in bone diseases[2]. In the presence of secondary causes, the potential for bone growth is unaltered, but there are factors that prevent this potential from being expressed, including malnutrition and systemic diseases[2].

Celiac disease is characterized by permanent gluten intolerance in people who are genetically susceptible. Gluten provokes an inflammatory reaction that damages the villi in the small intestine, causing an inadequate absorption of nutrients. The disease has a varied spectrum of presentations, ranging from the classic form (chronic diarrhea, abdominal pain and distension, weight loss, failure to thrive and signs of malnutrition) to atypical and silent forms with no gastrointestinal symptoms[3,4].

A great deal of research has been carried out into short stature in isolation as an atypical form of presentation of celiac disease. Studies have reported varying frequencies (from 1.7 to 59.1%), depending on the selection criteria adopted, the study location and the diagnostic approach employed[5-13].

Screening patients with short stature for celiac disease is not part of the medical routine in our country, since these tests are expensive and not always available on the Brazilian National Health System (SUS, *Sistema Único de Saúde*). Notwithstanding, there is already consensus that children and adolescents with short stature should be serologically screened for celiac disease. This recommendation is included in guidelines published by the Pediatric Gastroenterology Department of the Brazilian Society of Pediatrics (SBP - *Sociedade Brasileira de Pediatria*) and by the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition[14].

The objective of this study was to determine the frequency of positive serological assay results in children and adolescents with short stature, selected at outpatients clinics affiliated with SUS in the city of Recife, using human anti-tissue transglutaminase (anti-tTG) as a screening test for celiac disease.

Methods

This was a cross-sectional, descriptive study carried out between April and September 2004 at the General Pediatrics Clinic at Instituto Materno-Infantil Professor Fernando Figueira (IMIP) and at the Growth and Development Clinic at Hospital das Clínicas, Universidade Federal de Pernambuco, Recife, northeast of Brazil.

Children and adolescents aged 2 to 20 years were defined as short stature cases if their heights were below the third percentile for their age and sex according to the height/age curves published by the National Center for Health Statistics (NCHS), 2000[15]. Patients were excluded if they were less than two years old, had been diagnosed with bone metabolism diseases, bone dysplasia, intrauterine growth restriction, dysmorphic syndromes, chromosome diseases, metabolite storage diseases, endocrine disorders (hypopituitarism, hypothyroidism, diabetes mellitus, Cushing's disease, hypogonadism), or chronic renal failure, or if they had been using oral, intravenous or intramuscular glucocorticoids for a period greater than eight days, amphetamines or methylphenidate.

All guardians were informed and agreed to participate in the study, and signed a free and informed consent form. There was only one refusal, because the child would not accept blood being taken. The study was approved by the Human Research Ethics Committee at IMIP.

Anthropometric data, weight and height, were measured with children unclothed, with no shoes or socks, using a digital balance accurate to 0.1kg and a wall-mounted stadiometer accurate to 0.1cm. After measurement, a structured questionnaire was administered covering socioeconomic and demographic aspects as well as complaints related to celiac disease (abnormal intestinal rhythm, abdominal pains, flatulence, recurrent aphthous ulcers, difficulty gaining weight and height, irritability, history of anemia, other cases of celiac disease in the family).

Blood for serology was collected by venous puncture into tubes with no anticoagulant, which were then centrifuged to separate serum. Samples were subdivided and frozen at -20º C, until laboratory tests were carried out. Initial screening was carried out using anti-tTG assays; where these were positive, anti-endomysial antibody (AEA) was assayed as well.

Enzyme immunoassay (Biosystems, Spain) was used to determine IgA tTG using microplate tests. Samples with concentrations >20U/mL were defined as positive[16]. Indirect

immunofluorescence was used to determine AEA, using histological sections of distal monkey esophagus fixed on microscope slides as substrate (Biosystems, Spain). Uniform fluorescence in 1/5 saline solution dilution was defined as positive. Patients with positive anti-tTG serology were referred to the gastroenterology clinic to continue investigation of celiac disease.

Data were stored in Epi-Info version 6.0. Seropositivity was calculated as the proportion of individuals in the sample with positive serology.

Results

A total of 78 patients were evaluated between April and September of 2004; 41 (53%) were female and 37 (47%) were male. Median age was 9 years (P25=5 years, P75=12 years). Forty-five (58%) of the 78 study participants came from Recife and the metropolitan area, while 33/78 (42%) lived in provincial parts of the state of Pernambuco. Among the patients included, 72% came from families with a monthly income of two times the minimum monthly wage or less, and approximately 63% of the guardians had not completed primary education.

Seventeen (22%) of these children were classed as underweight on the basis of the relationship between body mass index (BMI) and age, i.e., they were below the 5th percentile of the reference standard. With relation to complaints, 58/78 (74%) of the mothers said their children had difficulty gaining weight, 67/78 (86%) reported failure to thrive and 47/78 (60%) of the children had a history of anemia.

The anti-tTG assays were positive in 3.8% of cases (3/78). These patients had AEA assayed as well, and one of them resulted positive. The patient who was positive for AEA had also had the highest anti-tTG concentration. Based on both anti-tTG and AEA being positive, the rate of seropositivity was 1.3%.

Clinical and laboratorial characteristics of the anti-tTG-positive patients are given in Chart 1.

Discussion

This study was carried out at teaching hospitals affiliated with SUS. IMIP is a philanthropic hospital, and Hospital das Clínicas belongs to Universidade Federal de Pernambuco. The great majority of people treated at both hospitals come from deprived populations.

The rate of seropositivity for anti-tTG was 3.8%, while AEA was only positive in the patient who showed the highest anti-tTG concentration. The administration of two serological tests in series contributed to refining diagnostic probability. It is important to point out that, to date, diagnosis of celiac disease is still based on observation of histological abnormalities; biopsy is an invasive and expensive method which is not appropriate for initial investigation[14]. Furthermore, the wide spectrum of celiac disease and its nonspecific clinical manifestations make it difficult to identify patients who require biopsy[14]. Over recent years, attempts have been made to find other diagnostic methods with good sensitivity and specificity for the screening and diagnosis of celiac patients.

The anti-tTG assay emerged as a great hope for celiac disease screening, since it is an easily-executed test with a relatively low cost and can be used in screening studies, with similar results to those obtained using AEA, which is considered the best serological test for this disease[14,16-18]. The AEA takes longer, costs more and is operator-dependent, which can lead to errors[14,17,19,20].

Chart 1 – Physical and laboratorial characteristics and signs and symptoms of anti-tTG-positive patients with short stature

Sex	Age	Height	BMI	Anti-tTG	AEA	Signs and symptoms
Fem	7 years, 9 months	112.7cm	Between P10-P25	44.167	Negative	Occasional abdominal pain, difficulty gaining weight and height. Prior history of anemia.
Fem	12 years, 10 months	139.5cm	<P5	55.065	Negative	Difficulty gaining weight and height. Prior history of anemia.
Fem	11 years, 8 months	129.0cm	Between P10-P25	152.007	Positive	Frequent abdominal pain, difficulty gaining weight and height. Prior history of anemia.

BMI: body mass índex; AEA: anti-endomysial antibody.

Based on the available evidence and on practical considerations, anti-tTG is the primary test recommended for screening[14,19]. The North American Society for Pediatric Gastroenterology, Hepatology and Nutrition[14] recommends the anti-tTG as the initial screening test for groups at risk of celiac disease, followed by intestinal biopsy. If histological findings are not consistent with celiac disease, it is recommended that the biopsy be re-evaluated by an experienced pathologist and that consideration be given to testing AEA, asssaying human leukocyte antigen (HLA) or repeating the biopsy.

Several different studies have compared AEA with anti-tTG in terms of sensitivity and specificity and have concluded that they are similar[21]. In patients with little or no symptomology, both tests offer a positive predictive value (PPV) of 75-80%, approaching 100% in symptomatic patients[14,21,22].

Several different studies[14,20,21,23-25] have shown that the accuracy of serological tests may not be as good in clinical practice as research suggests. Some authors have related AEA positivity with the degree of villous atrophy rather than with clinical symptomology, which may reduce the number of cases positive for celiac disease, particularly where villous atrophy is less severe[14,25,26]. Seronegative cases of celiac disease do occur; these patients have a clinical presentation with symptoms and response to gluten-free diets similar to those observed in seropositive patients[25].

Some authors[20,22] recommend the performance of serial serological tests before indicating biopsy: first, anti-tTG; when positive, confirmation using AEA. In a recent article, Barker *et al*[27] suggested using anti-tTG as a screening test. These authors indicate the performance of biopsies in children with levels >20U/mL and <100U/mL, since diagnostic precision is limited within this range. Values >100U/mL were associated with histological abnormalities caused by celiac disease, and the authors do not believe that these patients require biopsy, thus reducing costs[27]. While this is an important suggestion, particularly for locations where resources are scarce, the study requires further investigation and its reproducibility must be confirmed.

Celiac disease used to be considered rare in Brazil, and there was a scarcity of studies into its prevalence. It is only in recent years that short stature has come to be investigated as a clinical presentation of the disease[6,28-30]. Queiroz *et al*[6] found a celiac disease prevalence of 4.7% among patients with short stature who had already undergone in-depth investigation at a specialized center.

Among low-income populations, short stature is very often attributed to living conditions and chronic malnutrition. The effect of environment on growth is well established[31-34]. It is known that unhealthy living conditions and chronic malnutrition are negative stimuli and that the malnutrition caused by poverty is most obviously manifest in failure to thrive[32,33]. Celiac disease also affects these patients and may aggravate malnutrition. In a study carried out at IMIP, seroprevalence of celiac disease was 1.9%, based on positive anti-tTG and AEA antibodies[35]. The fact that that study was carried out at a pediatric hospital which is a center of excellence in the state of Pernambuco may have introduced a prevalence bias, since at these services there is a greater probability of undiagnosed patients under investigation for clinical conditions compatible with celiac disease (anemia, short stature, and abdominal pains)[35].

Celiac disease is a cause of short stature that should not be forgotten, particularly in deprived populations, and must be borne in mind during diagnostic investigations. It is important to point out that serological tests are not performed as part of the SUS service in Pernambuco, which impacts negatively on diagnosis. Considering that anti-tTG assays identify IgA antibodies, it is important to confirm serum IgA levels in patients with clinical signs compatible with celiac disease and negative serology. A small intestine biopsy is an indispensable part of the sequence of diagnostic investigation of seropositive patients[36].

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