World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1930

World J Gastroenterol 2013 March 28; 19(12): 1930-1935
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Decreased prevalence of celiac disease among Brazilian elderly

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Received: June 21, 2012 Revised: December 3, 2012
Accepted: December 12, 2012
Published online: March 28, 2013

Abstract

AIM: To evaluate the prevalence of celiac disease in a group of Brazilian individuals over 60 years of age and compare it with the previously known prevalence in a pediatric group living in the same geographical area.

METHODS: The research protocol was approved by the Ethics Committee of the University of Brasilia School of Medicine, Brasilia, Brazil. Blood samples from 946 individuals (295 male and 651 female) aged 60 years or older were collected between May 2010 and July 2011. The study subjects' mean and median ages were 68.1 and 67 years, respectively, ranging from 60 to 92 years. That age distribution closely corresponded to the age distribution of the Brazilian population according to the Brazilian 2010 census. The participants were consecutive and unselected outpatients undergoing blood tests at the University of Brasilia Hospital's Clinical Pathology Laboratory. All sera were tested for immunoglobulin A anti-transglutaminase antibodies (IgA-tTG) by enzyme-linked immunosorbent assay, and those that were positive were further tested for immunoglobulin A anti-endomysium antibodies (IgA-EMA). Human leukocyte antigen (HLA) genotyping was performed for all individuals who exhibited positive serologic results for IgA-tTG and/or IgA-EMA.

RESULTS: Out of the 946 studied patients, only one previously diagnosed case of biopsy-proven celiac disease was detected. For the remaining subjects, nine serum samples tested positive for IgA-tTG antibodies; however, none of them tested positive for IgA-EMA antibodies. The HLA genotyping of those nine subjects revealed that one was carrying DQA1*0501 and two were carrying DQB1*0201 alleles. These data showed that, among those 946 elderly individuals, the prevalence of celiac disease (CD) was 0.1% (95%CI: 0.00-0.59). The prevalence of CD for the elderly group was compared with that observed for the group of 2034 children younger than 15 years (age range, 1-14 years; mean age, 8 years) who took part in our previous CD prevalence screening study. All the children came from the same geographical region and shared a similar ethnic and low-income background. As in the elderly group in

the current study, the younger group was made up of consecutive outpatients who underwent blood evaluation at the University of Brasilia Hospital's Clinical Laboratory. The prevalence of biopsy-proven CD among those children was 0.54% (95%CI: 0.27-0.57). The comparative analysis between the two groups resulted in the following values: odds ratio = 0.19 (95%CI: 0.01-1.45) Fisher test $P = 0.06$.

CONCLUSION: The prevalence of CD among the children of our previous study was 5.4 times higher than that found in the present elderly group.

Key words: Celiac disease; Gluten-sensitive enteropathy; Epidemiology; Elderly; Mortality

Almeida LM, Castro LC, Uenishi RH, de Almeida FC, Fritsch PM, Gandolfi L, Pratesi R, de Medeiros Nóbrega YK. Decreased prevalence of celiac disease among Brazilian elderly. *World J Gastroenterol* 2013; 19(12): 1930-1935 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1930.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1930

INTRODUCTION

Celiac disease (CD) is a chronic autoimmune-mediated disease with both intestinal and systemic manifestations that are induced by the ingestion of gluten in genetically predisposed individuals. CD-related intestinal abnormalities are mainly characterized by villous atrophy, crypt hyperplasia, and lymphocyte infiltration of the small mucosa caused by T-cell responses to the enzyme transglutaminase 2[1] and gluten-derived gliadin peptides[2]. CD is a lifelong disease that can start at any age. As it involves multiple organs or systems, it may manifest in a wide range of clinical pictures. The only effective therapy for CD is strict dietary abstinence from gluten-containing foods.

During the last few decades, the advent of reliable serologic tests has greatly facilitated in the diagnosis of CD, allowing large-scale screening studies to be performed. Worldwide prevalence rates, determined by a similar sequential testing paradigm [*i.e.*, immunoglobulin A anti-transglutaminase (IgA-tTG) antibodies and/or anti-endomysium antibodies (IgA-EMA) tests] averaged 1:160 in the Western world[3]. Recent epidemiological data showed that CD is also a common disease in developing countries (Middle East, South Asia, Africa, and South America), with a prevalence similar to that in Western countries[4]. The prevalence of CD in Brazil has shown significant variation, probably due to the different degrees of miscegenation of the ethnic groups that make up the Brazilian population, especially Amerindians, Afro-descendants, and Europeans. Screening studies performed during the last decade in distinct Brazilian regions showed prevalence rates varying from 1:214 to 1:681 in presumably healthy blood donors[5-8], and from 1:119 to 1:417 in the general population[9,10]. The geographical variation in the prevalence rates may also be due to differences in genetic background, age-related differences of exposure to gluten, and/or to changes in environmental risk factors. Due to the widely variable pattern of its clinical spectrum, the confirmatory diagnosis of CD can be delayed, after being left unrecognized for many years[11,12].

Traditionally, CD has been regarded as a disease of childhood and early adulthood that rarely develops in older people. Nevertheless, during the last few decades, diagnosis of CD among adults and the elderly has increased, including patients 70 years of age and older. CD is considered a lifelong disease and consequently a progressive increase in its prevalence would be expected in older age groups.

Nevertheless, studies focusing on this topic are rather controversial. While some of the studies pointed to a high prevalence of CD in older age groups[13,14], others showed a higher prevalence in children and adolescents[10,15,16].

In a previous study, we found an increased prevalence of CD in children compared with adults and elderly individuals[10]. Following the same line of research, in this study we aimed to determine the prevalence of CD in elderly Brazilians over 60 years of age, all of them living in the same geographic region and belonging to similar socioeconomic and cultural backgrounds as the children evaluated in our previous study.

MATERIALS AND METHODS

The research protocol was approved by the Ethics Committee of the University of Brasilia School of Medicine. All individuals included in the protocol were informed about the objectives, related risks, and benefits of this study, and agreed with the use of the collected specimens for research. Between May 2010 and July 2011, a total of 946 outpatients (295 male and 651 female) aged 60 years or older had a blood sample collected and stored at -20 ℃ until their use. The subjects' mean and median ages were 68.1 and 67 years, respectively, ranging from 60 to 92 years. That age distribution closely corresponded to the age distribution of the Brazilian population according to the 2010 census[17].

The participants were consecutive and unselected outpatients undergoing blood tests at the Clinical Pathology Laboratory of the University of Brasilia Hospital. The most frequent reasons for blood testing were routine health check-up, suspected or recurrent infections, chronic ailments, metabolic disorders, and pre-operative check-up. Patients from the gastroenterology outpatient clinic were excluded to avoid a selection bias. No other exclusion criteria were applied, regardless of the possible existence of symptoms or conditions commonly associated with CD.

The University of Brasilia Hospital is a public reference hospital that predominately serves a low-income population from the city of Brasilia and the surrounding area in the midwest region of Brazil. Such individuals usually depend on the Governmental National Health

System. They exhibit mixed ancestry, with a considerable contribution of European intermixed with variable parcels of other races, mainly Afro-descendants and Amerindians.

The serum samples from the patients were tested for IgA-human anti-tissue-transglutaminase-IgA (htTG) antibodies using an IgA-htTG enzyme- linked immunosorbent assay Kit (QUANTA Lite® h-tTG IgA Inova Diagnostic, Inc. San Diego, CA, United States). The limit of positivity was set at 20 arbitrary units, in accordance with the manufacturer's instructions. As a second step, all IgA-htTG positive samples were further tested for the presence of IgA-EMA antibodies using indirect immunofluorescence on primate distal esophagus cryostatic sections (Inova Diagnostic, Inc. San Diego, CA, United States).

All individuals who exhibited positive serologic results for IgA-tTG and/or IgA-EMA antibodies underwent HLA genotyping. Genomic DNA was extracted from peripheral venous blood samples using the Illustra ™ blood genomicPrep. Mini Spin Kit (GE Healthcare, Buckinghamshire, United Kingdom). HLA-DQA1*0501 (DQ2 α chain), HLA-DQB1*02 (DQ2 β chain), HLA-DQA1*0301 (DQ8 α chain), and DQB1*0302 (DQ8 β chain) genotyping was performed by polymerase chain reaction amplification using sequence-specific primers (PCR-SSP). For internal positive amplification control, each PCR reaction included a primer pair for a conserved region of the *DRB1* gene. The amplified products were separated using 2% agarose gel, stained with ethidium bromide and then visualized under an ultraviolet transilluminator.

RESULTS

Out of the 946 subjects, only a single previously diagnosed case of biopsy-proven CD in a 66-year-old woman was detected. Among the remaining subjects, nine serum samples tested positive for IgA-tTG antibodies. None of the patients tested positive for IgA-EMA antibodies. HLA genotyping disclosed the presence of one CD predisposing allele in three of the IgA-tTG positive elderly. The clinical and laboratory data of the nine patients who tested positive for IgA-tTG antibodies are depicted in Table 1. These data showed that among those 946 elderly individuals, the prevalence of CD (n = 1) was 0.1% (95%CI: 0.00-0.59).

The prevalence of CD for the elderly group was compared with that observed for the group of 2034 children younger than 15 years (age range, 1-14 years; mean age, 8 years) who took part in our previous CD prevalence screening study[10]. All the children came from the same geographical region and presented a similar ethnic and low-income background. As in the elderly group in the current study, the younger group was made up of consecutive outpatients who underwent blood evaluation at the University of Brasilia Hospital's Clinical Laboratory. The prevalence of biopsy-proven CD (n = 11) among those 2034 children was 0.54% (95%CI: 0.27-0.57).

DISCUSSION

Out of the 946 elderly individuals tested in this study, only a single case of previously-detected CD was found. Although nine individuals showed moderately increased levels of anti-tTG antibodies ranging from 30.6 to 52.3, no subjects tested positive for IgA-EMA antibodies. Although IgA-tTG is an effective screening test for CD, occasional anti-tTG false positive results cannot be excluded, especially in the presence of other autoimmune diseases[18,19]. The clinical effectiveness of the IgA-tTG test is improved if its positive results are confirmed with the IgA-EMA test[20] and by the presence of predisposing alleles on HLA PCR-SSP typing. Typing for HLA-DQ2 and HLA-DQ8 is a useful tool for either excluding CD or making its diagnosis unlikely in the case of a negative test result for both markers[21,22]. Predisposing HLA alleles were present in only three of the subjects who tested positive for IgA-tTG antibodies. A jejunal biopsy was suggested and refused by both patients carrying the higher degree of risk allele DQB1*0201, although they agreed in being followed with periodical clinical evaluations and serological testing.

The results of this current screening are in agreement with the result obtained in our previous study, in which an unanticipated variation in the prevalence of CD was found[10]. In this study performed in the same geographical area with a similar population group, most cases of CD were clustered in the younger age group, with the prevalence of CD in children aged 1 to 14 years being 2.6 times higher than the one found for adults and elderly individuals (5.44 *vs* 2.11 per 1000, respectively). This variation in the prevalence of CD between different age groups was actually unexpected, considering that intestinal sensitivity to gluten is a permanent condition. Aside from gluten ingestion, CD might be triggered at any stage of life by additional environmental factors that remain largely unknown. Thus, a progressive increase in prevalence rates towards advanced ages or, at least, a similar rate throughout life would be expected. Recent studies in Finland by Vilppula *et al*[13,14] showed an increase in the prevalence of CD among individuals over 52 years of age compared with the general prevalence in Finnish children (2.13% *vs* 1%). Furthermore, the authors also demonstrated an increasing prevalence throughout a three-year period for the same group, going from 2.13% to 2.34%, and resulting in an annual CD incidence of 0.08% in that population. However, several other studies report contradictory results by showing a higher prevalence of CD in younger populations[15,16,23,24].

Several hypotheses have been suggested to explain this discordance in the prevalence rates among different age groups, although none have been definitely proven. One hypothesis offered to justify a higher prevalence in the younger age group is that the incidence of CD, similarly to other autoimmune diseases, is progressively

Table 1 Clinical and laboratory data of patients who tested positive for immunoglobulin A anti-transglutaminase antibodies by enzyme-linked immunosorbent assay

Patient	Sex	Age (yr)	tTG	EMA	HLA	Symptomatology and associated disorders
1	M	63	39.9	Neg	Negative	Anemia, arthritis
2	M	71	34.5	Neg	DQB1*0201	Hyperthyroidism
3	M	81	31.3	Neg	Negative	No complaints
4	F	60	42.9	Neg	DQB1*0201	No complaints
5	F	60	30.6	Neg	Negative	Osteopenia, arthritis, recurrent abdominal pain, flatulence
6	F	63	52.3	Neg	Negative	Arthritis, osteoporosis, hyperthyroidism
7	F	65	34.0	Neg	Negative	No complaints
8	F	68	45.3	Neg	DQA1*0501	Osteoporosis, weight loss, flatulence
9	F	72	45.2	Neg	Negative	Osteoporosis

M: Male; F: Female; Neg: Negative; tTG: Transglutaminase; EMA: Endomysium; HLA: Human leukocyte antigen.

increasing worldwide. CD was considered a rare disease until the late 1970s, and its prevalence was estimated to be as low as 0.03%[25]. With the advent of highly sensitive and specific serological tests, a dramatic rise in its prevalence was observed during the following decades. However, this increase would not be solely due to better diagnostic methods that enabled extensive screening studies and diagnosis of atypical forms of the disease; consistent with other autoimmune disorders that have shown rising incidence rates over the last few decades[26], CD would also have shown a significant increase in its prevalence, consequently justifying the increased number of cases found among younger age groups[27,28]. Although the causes underlying this increased age-related prevalence remain unknown, likely explanations include environmental influences (such as changes in quantity and quality of cereal processing), changed patterns of early childhood exposure to infectious agents that impair the natural development of the immune system (hygiene hypothesis)[29], and changes in infant dietetic habits[30].

Another possible cause for an increased CD prevalence in the younger population was suggested by Mariné *et al*[15], who proposed that a significant number of CD cases that appear during childhood progress to a latent form or into total gluten tolerance. Those patients would therefore exhibit negative serologic results as they got older. Several other studies support this hypothesis[31-33], although the number of described spontaneous recoveries of normal villous architecture in CD patients on a gluten-containing diet is generally small[34] and it is uncertain as to whether these clinically silent periods accompanied by negative serologic tests can be considered only temporary remissions or actual definitive recoveries. Extended follow-up is therefore required for these patients[32,35].

A third explanation is the postulated existence of an increased mortality rate among CD patients. Publications addressing this issue are numerous, but conflicting[28,36,37]. The reported overall mortality rates among CD patients vary from 1.26%[29] to 3.9%[19] in studies focusing on undiagnosed CD in adults. Despite the differing results, at least two publications point to undiagnosed CD as a major cause of increased morbidity and mortality among individuals with the disease[28,38].

These three hypotheses are not mutually exclusive, and it is both possible and probable that each of these factors contribute, to a greater or lesser degree, to the observed variation in the prevalence of CD in elderly individuals, depending on the different environmental conditions found in distinct regions or countries.

In Brasilia, many of the low-income adult population came from poor regions of the country, where they have had little or no access to medical care during childhood, and awareness of CD was somehow deficient among healthcare professionals. In these regions, CD knowledge several decades ago was not much different from that before effective treatment for CD was established. Even with the current facilities for the proper diagnosis and compliance of CD patients, diet behaviors among individuals of economically disadvantaged backgrounds in Brazil is still far from ideal.

In Finland, the higher prevalence of CD in patients aged over 52 years and the lower CD mortality rate[13,14] are noteworthy. Comparisons between Finland and Brazil are contentious at best, since they occupy different positions in the World Health Organization's health system ranking of countries[39] (31st and 125th, respectively). If this same survey was again performed in Brasilia, but instead focused on a higher socioeconomic group with a higher quality of life, the outcomes would probably have been different.

Our study has potential limitations that should be noted. The screening of the elderly group was conducted in a single geographic setting and the participants were unselected outpatients undergoing routine blood tests, although both groups, children and elderly, were similar with regard to their ethnicity and socioeconomic level. In addition, the number of elderly screened was insufficient to reach statistical significance ($P = 0.06$). In spite of these drawbacks, this study supports our previous findings of an age-related variation in the prevalence rate of CD. Only a single biopsy-proven previously diagnosed case of CD was identified among the elderly group, showing that the prevalence of CD among the children of our previous study was 5.4 times higher than that found in the present elderly group. These findings reinforce that, for the low socioeconomic populations

of our region, prevalence of CD is unexpectedly higher in children compared to older individuals, and that this discrepancy increases towards older ages. We hypothesize that among the plausible explanations for the discrepancy between the CD prevalence rates among children and elderly, the most likely culprit would be an increased mortality rate among undiagnosed celiac disease patients.

COMMENTS

Background

Epidemiological studies during the last few decades have shown an increasing prevalence of celiac disease over time, not only in Europe and in people of European ancestry, but also in developing countries. Socioeconomically disadvantaged population groups in developing countries additionally suffer from a low level of awareness, clinical suspicion of this disorder among physicians, and difficult access by the patient to diagnostic methods, which result in unrecognized or delayed diagnosis of celiac disease (CD). Additionally, patients in whom an appropriate diagnosis is reached have their treatment hampered by an impossibility to use commercial gluten-free products, which are too expensive for these populations, as well as the lack of patients' awareness and information regarding their diet gluten content.

Research frontiers

Overcoming the difficulties of treating CD in the context of developing countries implies the introduction of CD diagnostic laboratory tests as routine in the State Health System, funding research of cheap sources of gluten free foods, and an increased recognition of CD and of its symptoms.

Applications

The results obtained in this study may provide support for future epidemiological studies to map the onset of CD in different age groups in at-risk populations in developing countries. It can additionally contribute to the adoption of public health policies that will allow the socioeconomically disadvantaged access to health services for medical consultations, laboratory tests and, after diagnosis, financial support for a lifelong gluten-free diet.

Peer review

In this interesting survey, the authors report their results on the prevalence of celiac disease in a typical Brazilian population. The manuscript is very well written. The abstract is appropriate in length and content. The results are reported clearly. The discussion is exhaustive and provides an interesting view of this controversial topic.

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