Celiac disease in native Indians from Brazil: A clinical and epidemiological survey

Shirley Ramos da Rosa Utiyama, PhD1, João Luis Coelho Ribas, M.Cs1, Renato Mitsunori Nishihara, M.Cs1, Lorete Maria da Silva Kotze, MD, PhD2, Iara José de Messias-Reason, PhD1

1Laboratory of Immunopathology, Clinical Hospital, Federal University of Paraná, Curitiba, Paraná, Brazil.
2Cajuru Hospital, Pontifical Catholic University of Paraná, Curitiba, Paraná, Brazil.

Abstract

Background: Celiac disease has been described in populations from around the world, with recent data emphasizing the occurrence of the disease in ethnic minorities. There are only a few studies evaluating celiac disease in native Indians. Aims: This study aimed to screen the anti-endomysial antibody (IgA-EmA) in Kaingang and Guarani Indians from southern Brazil, in order to establish a clinical serological evaluation of celiac disease in these individuals. Material and Methods: Serum samples from 321 individuals (125 male and 196 female; 4-86 years old) from Mangueirinha Indigenous Reserve, State of Parana, Brazil, and 180 non-Indigenous healthy individuals (62 male and 118 female; 2-81 years old) were analysed to the presence of anti-endomysial antibody class IgA by indirect immunofluorescence assay. Amongst the Indians, 158 were Kaingang, 98 Guarani and 65 of mixed race. Indians presenting complaints of diarrhea (N=12) were also evaluated to the IgG class of anti-endomysial antibody. Results: None of the individuals showed positive results either to IgA or IgG anti-endomysial antibodies. Conclusions: Although the results indicate an absence of celiac disease in Kaingang and Guarani Indians, the authors call attention to the importance of following up indigenous children or adults presenting gastrointestinal complaints or other symptoms related to the disease. Consideration should be given to the genetic background of these individuals, allied to the inter ethnic marriages and the changing habits or occupational activities, that have gradually introduced diseases previously not described in indigenous populations.

Keywords: Celiac disease, Kaingang Indians, Guarani Indians, Brazilian Indians, anti-endomysial antibodies.

Introduction

Celiac disease (CD) is one of the most common immune mediated disorders. It is a chronic enteropathy, with genetic, environmental and autoimmune components, triggered by the ingestion of wheat gluten and other related proteins. HLA-DQ2 is found in up to 95% of CD patients, and the HLA-DQ8 in most of the remaining patients [1, 2].

Clinically, CD varies greatly, and is now considered to resemble a multisystemic rather than a gastrointestinal disorder. It can be found in classical, atypical, silent and latent forms. While the classical form is characterized by chronic diarrhea and malnutrition, in the atypical form of CD extraintestinal features such as osteoporosis, infertility, anemia, short stature and neurological problems can also be found [3].

The development of accurate serological tests for CD over the last decades, such as the antiendomysial and anti-tissue transglutaminase antibodies provided a valuable tool for screening individuals, in as much as the general population as in CD high risk groups [4,5]. CD was considered to be of low-prevalence until the 1950s, but currently, due to the availability of high sensitivity and
specificity diagnostic tests, its prevalence has been shown to be much higher than previously thought.

CD has a worldwide distribution, being described in different ethnic groups from North and South America, Europe, south and west Asia, Australia and New Zealand [6, 7]. The disease is rare among Africans and not expected among populations with no HLA DQ2, like Chinese and Japanese, except in individuals presenting HLA DQ8 [2].

Although several autoimmune diseases have been described in native Indian populations from around the world, studies evaluating the occurrence of CD among those individuals are scarce. Araya et al. described clinical and serological features of CD in patients and relatives of a Chilean population carrying Amerindian traits [8]. In addition, Galvão et al. reported twenty cases of CD in North of Brazil in a mixed population (Europeans and local Indians) [9]. On the other hand, high prevalence and more severe disease, as well as increased comorbidity and mortality rates, were demonstrated in different native Indian populations for other autoimmune diseases [10,11], such as rheumatoid arthritis [10,12,13], systemic lupus erythematosus [14,15], primary biliary cirrhosis [16-18], systemic sclerosis [19,20] and type 1 diabetes [21], amongst others. Both genetic and environmental components have been considered as the main factors influencing the development of these autoimmune disorders in native Indians.

Although Kaingang and Guarani represent the two largest Amerindian tribes living in south Brazil and some areas of Paraguay and Argentina, they are both culturally and genetically distinct from each other [22-24]. While Kaingang are semi-nomads and cultured, the Guarani are nomads and preserve secular customs and the Tupy native language. The Guarani represent the biggest indigenous community in Brazil, with approximately 46.000 individuals.

Several studies have evidenced genetic differences between Kaingang and Guarani Indians, specially related to MHC genes, also when compared with other South American Indians and other ethnic groups, such as Europeans and Africans [25-28].

The general Brazilian population is known to present a high degree of miscigenation between Europeans, Africans and native Indians, with a predominance of European ancestry in the South. Recent data has shown prevalence for CD of 1:273 [29] to 1:417 [30] in this region. The present study aimed to screen the anti-endomysial antibody (IgA-EmA) in Kaingang and Guarani Indians from Southern Brazil, in order to evaluate clinically the occurrence of CD in these individuals.

**Materials and Methods**

A total of 321 Indians (196 female and 125 male), from Mangueirinha Reserve (latitude 25°56'28"S; longitude 52°10'32"O), in the state of Parana, South Brazil, were studied. Amongst them, 158 were Kaingang, 98 Guarani and 65 mixed races (Kaingang and Euro-Brazilian individuals). As a comparison group, 180 healthy, voluntary non Indian subjects, from European ancestry were included. All individuals belong to the same geographic area and were matched for age and sex. Demographic data of these groups are informed (Table 1).

| Groups          | Number | Sex (F/M) | Mean Age (Range) | Median |
|-----------------|--------|-----------|------------------|--------|
| Indians (total) | 321    | 196/125   | 26.9 (4-86)      | 21     |
| Kaingang        | 158    | 100/58    | 30.8 (4-80)      | 27     |
| Mixed           | 65     | 39/26     | 25.6 (4-86)      | 22     |
| Guarani         | 98     | 57/41     | 21.6 (5-86)      | 17     |
| Non-Indians     | 180    | 118/62    | 25.0 (2-81)      | 19     |

F: Female; M: Male, Mixed: Kaingang and non-Indian.

A structured questionnaire was used to collect socio-demographic data and symptoms and signs related to diseases. All Indian individuals presented good health conditions on blood collection and clinical evaluation. Some features related to their occupational activities, individual customs and some complaints or symptoms were summarized (Table 2).

| Individual customs | Kaingang % (N) | Mixed % (N) | Guarani % (N) |
|--------------------|----------------|-------------|---------------|
| Alcohol users      | 13.4 (21/158)  | 7.7 (5/65)  | 14.3 (14/98)  |
| Tobacco users      | 32.3 (51/158)  | 12.3 (8/65) | 31.6 (31/98)  |
| Drugs users        | 0 (0/158)      | 3.1 (2/65)  | 1.0 (1/98)    |
| Tatoo              | 1.26 (2/158)   | 0 (0/65)    | 3.06 (3/98)   |
| Agricultural       | 18.3 (29/158)  | 13.9 (9/65) | 5.3 (5/98)    |
| Student            | 27.8 (44/158)  | 24.6 (16/65)| 46.9 (46/98)  |
| Homemaker          | 38.6 (61/158)  | 27.7 (18/65)| 27.5 (27/98)  |
| Complaints         | 27.2 (43/158)  | 29.2 (19/65)| 30.6 (30/98)  |
| Diarrhea           | 1.3 (2/158)    | 6.1 (4/65)  | 6.1 (6/98)    |

Mixed: Kaingang and non-Indian.

The study was approved by the ethical committee of the Clinical Hospital of the Federal University of Parana, National Council of Research (CONEP), National Foundation of Indians (FUNAI) and by the local leaders of the tribes.

Anti-endomysial antibodies (IgA-EmA) were evaluated in all serum samples, by indirect immunofluorescence assay (FITC), according to Volta et al. [31]. Cryostatic sections of human umbilical cord were used as substrate and FITC anti-IgA human as conjugate (Inova, San Diego, USA). Sera were considered positive if fluorescence was observed at a dilution of 1:2.5, and titred up to the
end-point. Positive and negative controls were used for each batch. Similarly, Indian individuals presenting complaints of diarrhea (N=12; Table 2) were also evaluated to the anti-endomysial antibodies class IgG (IgG-EmA), using FITC anti-IgG human conjugate (Dako, Denmark).

Statistical analysis was performed with Statistical (Microsoft USA) software, using the Fisher exact test. The adopted level of significance was P<0.05.

Results
The serological screening for CD based on IgA-EmA, performed in the Kaingang and Guarani Indians as well as in the non-Indian subjects, is in table 3. None of the investigated individuals showed a positive result to the IgA-EmA. The analysis of IgG-EmA in those Indians who informed in the structured questionnaire the occurrence of diarrhea with frequency also showed negative results (Table 3).

| Table 3 Anti-endomysial antibodies in Indian and non-Indian individuals of Southern Brazil |
|---------------------------------|----------|----------|----------|----------|
|                                  | Kaingang (%) | Mixed (%) | Guarani (%) | Non-Indians (%) |
| IgA-EmA                         |           |          |            |              |
| Positives                       | 0 (0)     | 0 (0)    | 0 (0)      | 0 (0)        |
| Negatives                       | 158 (100) | 65 (100) | 98 (100)   | 180 (100)    |
| IgG-EmA                         |           |          |            |              |
| Positives                       | 0 (0)     | 0 (0)    | 0 (0)      | 0 (0)        |
| Negatives                       | 2 (100)   | 4 (100)  | 6 (100)    | -            |

EmA: Endomysial antibody, Mixed: Kaingang and non-Indian

Discussion
The population of native Indians in Brazil at the time of its discovery in 1500 was approximately 6 million individuals. However, recent data estimates this number to be only 460000 [32]. During the centuries, millions of native Indians were exterminated due to the colonization process, slavery and diseases.

This is the first report of serological and clinical evaluation of CD in Kaingang and Guarani Indians from southern Brazil. The results showed an absence of CD in these native indigenous, as well as in the control group of non-Indians from southern Brazil. None of the individuals were positive to IgA-EmA, although approximately 6% of Guarani and Mixed group had complaints of diarrhea (Table 2). This group of individuals was also negative for IgG-EmA (Table 3). Probably the high frequency of intestinal parasitosis and lack of nutritional diets of some Indians may be the cause of diarrhea in these individuals to subjects [33, 34]. The presence of other autoimmune disease markers like rheumatoid factor and anti-nuclear antibodies, in association with clinical manifestations of rheumatic diseases, as to was previously described in these individuals by the authors [35], who observed complaint of arthralgy in almost 30% of the subjects (Table 2). Similarly, Utiyama et al. [36] observed the occurrence of autoantibodies in Kaingang and Guarani Indians from Rio das Cobras and Ivai Indigenous Reserve in South Brazil.

Recent reports have showed the occurrence of CD among ethnic minorities and therefore there was reason to believe that this disease be under-diagnosed in those groups, as described by Brar et al. [37] in African-American patients. Although the disease has been considered uncommon in India until recently, Sood et al. observed a prevalence of 1:310 in school children of the Punjab, showing that CD is not rare in wheat-eating areas of North India [38]. Similarly, Freeman described CD in 14 adult Asian individuals from a single Canadian teaching hospital, living in North America, including 10 patients from Punjab, two from Japan and one from China [39]. In addition, Butterworth reported clinical and laboratorial features on 40 CD patients of South Asian origin living in the United Kingdom [40]. According to the authors, special attention needs to be given to methods that encourage adherence to gluten-free diet in affected individuals of minority groups [37, 40].

Interestingly, the differences in HLA alleles observed in CD patients from South Asia when compared with Caucasian patients, suggested that non-HLA genes play a stronger role in the susceptibility and presentation of the disease in the former group [41].

Analyses of HLA-DQA1 and HLA-DQB1 class II gene polymorphism in Kaingang and Guarani Amerindians from southern Brazil, showed four different DQA1-DQB1 haplotypes:DQA1*0401-DQB1*0402,DQA1*0501-DQB1*0301, DQA1*03-DQB1*0302 and DQA1*03-DQB1*0302 [24]. In this context, considering that CD is closely associated with genes that code for HLA-DQ2 in 95% of the patients (DQA1*05/DQB1*02) and HLA-DQ8 in most of the remainder (DQA1*03/DQB1*0302) [2], it is reasonable to suppose a certain degree of susceptibility to CD in these investigated populations, especially in the Mixed group, due to the inter ethnic marriages that have occurred in the last centuries.

In addition, the gradual change in the traditions, cultural practices and nutritional habits amongst Indians have introduced several factors that might represent a risk to autoimmune diseases in this population. Among these, it must be emphasized the ingestion of wheat gluten and related cereal proteins (rye, barley) by individuals whose nutrition was formerly based on foods, such as fruits, meat and fish.

Although none of the Indian individuals analyzed in the present study showed positivity to EmA-IgA, the authors consider it important that physicians who attend indigenous populations stay alert and follow up children or adults that present persistent gastrointestinal complaints or other symptoms related to CD. The genetic background, allied to hormonal and immunological factors, besides the changing of habits or even occupational activities, may gradually lead to the occurrence of diseases previously not seen in these native populations, as observed in other
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References

1. Alaeddini A, Green PH. Narrative review: celiac disease: understanding a complex autoimmune disorder. Ann Intern Med 2005; 142 (4): 289-298.
2. Bai J, Zeballos E, Fried M, et al. Celiac Disease. World Gastroenterol News 2005; 10(2): 1-8.
3. Lo W, Sano K, Lebwohl B, Diamond B, Green PH. Changing presentation of adult celiac disease. Dig Dis Sci 2003; 48: 395-398.
4. Kotze LM, Utiyama, SRR, Oliveira NP, Fiedler PT, Messias-Reason IT. Celiac disease in children and adolescents with Down Syndrome. J Pediatr 2005; 81(5): 373-376.
5. Nishihara RM, Kotze LMS, Utiyama, SRR, Oliveira NF, Fiedler PT, Messias-Reason IT. Celiac disease in children and adolescents with Down Syndrome. J Pediatr 2005; 81(5): 373-376.
6. Fasano A, Catassi C. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. Gastroenterol 2001; 120(3): 636-651.
7. Green PHR, Jabri B. Coeliac disease. Lancet 2003; 362(2): 383-391.
8. Araya M, Mondragón A, Pérez-Bravo F, et al. Celiac disease in a Chilean population carrying Amerindian traits. J Pediatr Gastroenterol Nutr 2000; 31(4): 381-386.
9. Galvão LC, Gomes RC, Ramos AMO. Doença Celíaca: relato de vinte casos no rio Grande do Norte. Arq Gastroenterol 1992; 29(1): 28-33.
10. Jacobsson LT, Knowler WC, Pillemer S, et al. Rheumatoid arthritis and mortality. A longitudinal study in Pima Indians. Arthritis Rheum 1993; 8(36): 1045-1053.
11. Arbour L, Field L, Ross P, Eriksen A, Yoshida E. The mystery of primary biliary cirrhosis in British Columbia’s First Nations people. Int J Circ Health 2004; 63(2): 185-188.
25. Belich MP, Madrigal JA, Hildebrand WH, et al. Unusual HLA-B alleles in two tribes of Brazilians Indians. Nature 1992; 28: 326-329.
26. Tsuneto LT, Probst CM, Hutz MH, et al. HLA class II diversity in seven Ameridian populations. Clues about the origins of the Ache. Tissue Antigens 2005; 6(62): 512-526.
27. Guerra LK, Rehfeldt IR, Lima MT, Petzl-Erler ML, de Messias IJ. BF and C3 genetic polymorphism in Kaigang Indians from southern Brazil. Hum Hered 1992; 42(3): 153-156.
28. Messias IJT, Erler, ML, Rehfeldt IR, Lima MT, Guerra LK. Variability of Bf and C3 among Guarani Indians from Southern Brazil. Rev Bras Genetica 1993; 16(3): 803-809.
29. Melo SB, Fernandes MI, Peres LC, Troncon LE, Galvão LC.. Prevalence and demographic characteristics of celiac disease among blood donors in Ribeirão Preto, state of São Paulo, Brazil. Dig Dis Sci 2006; 51(5): 1020-1025.
30. Pereira MA, Ortiz-Agostinho CL, Nishitokukado I, et al. Prevalence of celiac disease in an urban area of Brazil with predominantly European ancestry. World J Gastroenterol 2006; 12(40): 6546-6550.
31. Volta U, Molinaro N, de Franceschi L, Fratangelo D, Bianchi FB. IgA anti-endomysial antibodies on human umbilical cord tissue for celiac disease screening. Save both money and monkeys. Dig Dis Sci 1995; 40(9): 1902-1905.
32. FUNAI. Indigenous Nacional Foundation. Access in February 2007 at: www.funai.gov.br.
33. Navone GT, Gamboa MI, Oyhenart EE, Orden AB. Intestinal parasitosis in Mbya-Guarani populations from Misiones Province, Argentina: epidemiological and nutricional aspects. Cad Saude Publica 2006; 22(5): 1089-1100.