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ORIGINAL ARTICLE / *ARTIGO ORIGINAL*

Serologic screening of celiac disease in adolescents

Triagem sorológica para doença celíaca em adolescentes

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ABSTRACT: ***Objective:*** This study aimed to identify the seroprevalence of celiac disease in adolescents from public schools in the city of Salvador, Bahia. ***Methods:*** This was a cross-sectional study with probabilistic sample of 1,213 adolescents, aged 11 to 17 years old, of both genders. The body mass index was used to determine the participants' nutritional status based on the percentiles for age and gender recommended by the World Health Organization. Measurement of the anti-human transglutaminase immunoglobulin A (anti-tTG-IgA) antibody was established as the specific screening test for celiac disease, which involved an enzyme-linked immunosorbent assay (ELISA). Descriptive analysis was performed using proportions and means (standard deviation). ***Results:*** The female gender prevailed in the sample, and most of the participants had normal weights. The anti-tTG-IgA antibody was positive in 6/1,213 (0.49%) adolescents. ***Conclusion:*** The seroprevalence of celiac disease was 0.49% in the investigated adolescents. Further studies are necessary to establish the prevalence of celiac disease in this age range.

Keywords: Celiac disease. Adolescent. Anti-transglutaminase antibody. Seroepidemiologic studies. Population-based survey. Schoolchildren.

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RESUMO: ***Objetivo:*** Este estudo objetivou identificar a soroprevalência da doença celíaca em adolescentes de escolas públicas da cidade de Salvador, Bahia. ***Método:*** Trata-se de um estudo transversal com amostra probabilística de 1.213 adolescentes de 11 a 17 anos, de ambos os sexos. O índice de massa corporal foi utilizado para o diagnóstico do estado nutricional, adotando-se os percentis segundo idade e sexo, propostos pela World Health Organization. O anticorpo anti-transglutaminase humana da classe imunoglobulina A (anti-tTG-IgA) foi adotado como teste sorológico para triagem da doença celíaca e foi determinado pela técnica do ensaio imunoabsorvente ligado à enzima (ELISA). Foi realizada análise descritiva, utilizando-se a proporção e a média (desvio padrão). ***Resultados:*** O sexo feminino predominou entre os adolescentes, e a maioria encontrava-se com adequado estado nutricional. O anticorpo anti-tTG-IgA foi positivo em 6/1.213 (0,49%) adolescentes. ***Conclusão:*** A soroprevalência de doença celíaca entre os adolescentes estudados foi 0,49%. Novas investigações são necessárias para confirmar a prevalência de doença celíaca nessa faixa etária.

Palavras-chaves: Doença celíaca. Adolescente. Anticorpo anti-transglutaminase. Estudos soroepidemiológicos. Estudo de base populacional. Escolares.

INTRODUCTION

In the past, the celiac disease (CD) was considered rare and as mainly affecting the pediatric population[1]. Recently, this panorama has been changing, mainly due to the development of more sensitive and specific serological tests, which, besides favoring the early diagnosis, make possible the conduction of several screening inquiries in asymptomatic subjects whose results indicate that the real prevalence of CD may be higher than 1% in different places[1-5]. Lately, CD has been diagnosed especially in a later phase of life, and the highest prevalence is found in female adults[6].

It is known that the CD may appear with variable frequency in children and adults in several geographic areas, with a wide spectrum of symptoms, and may happen in subjects that do not present any symptoms[1-5]. In Western countries, there are records of CD reaching around 1% of the general population[6,7]. In the United States, a 0.71%[7,8] prevalence was identified and, in the European continent, the highest occurrence of CD was found in Finland (2.4 to 2.6%) and the lowest in Germany (0.3 to 0.5%)[6,9]. Studies have reported that the CD prevalence in developing countries is similar to that seen in the Western world, with the following data: Middle East (0.5 to 1.8%), East and South of Asia (0.32 to 1.04%), North of Africa (0.14 to 5.6%), and Latin America (0.15 to 2.7%)[9,10]. In Brazil, results of studies carried out in some regions showed that the CD prevalence is similar to that found in developed countries, varying from 0.15 to 1.94%[11-14].

Despite the advance in diagnostic techniques, it is possible to assume that most CD cases still remain undiagnosed. Results of studies indicate that the late diagnosis of CD raises the risk of complications and of the disease severity, besides increasing the chance of associated comorbidities installation, conditions that can be prevented with early diagnosis and treatment[4,15,16]. Even though CD is a severe disease, there are only few population-based studies performed regionally or nationally in Brazil about its occurrence. The objective of this study was to identify CD seroprevalence in healthy adolescents from public schools in Salvador city, Bahia, Brazil.

METHOD

Cross-sectional study that is part of a bigger investigation named "Psychosocial Factors as Elements that have an effect in Health, Nutrition and Cognitive Development Conditions of Elementary Students from Public Schools in Salvador/BA". It was carried out with 11 to 17 year-old students both male and female, of Elementary Levels (7th, 8th and 9th years) from public schools in Salvador city, Bahia, Brazil. For the sample calculation, information from the 2007 School Census was used, which were available by the Education Secretariat from Bahia State, resulting in 77,873 students enrolled in state schools in Salvador. The sampling technique of conglomerates in two stages was adopted, represented by schools (first stage) and classes (second stage). To investigate CD seroprevalence, sample outlining was based on the occurrence of 0.8% for CD seroprevalence in blood donators in the city of Ribeirão Preto, São Paulo state[17], with a 95% confidence interval (95%CI) and maximum admissible error of 0.6, therefore the estimated sample minimum number was of 1,204 adolescents.

Twenty-three of 207 state schools were randomly chosen to compose this study and, later, the raffle of three classes per school was performed. All students enrolled in the raffled classes were eligible to participate in the study. Those who presented their parents or responsible ones' authorization through the free informed consent participated in the investigation. This consent detailed the objectives, procedures and steps of the study. Pregnant and breastfeeding women and adolescents with physical problems that did not follow the inclusion criteria of the greater study were excluded. The bigger investigation included 1,496 students; in which 1,215 of them performed blood collection, registering an 8.08% loss (281 students). All adolescents presented a written authorization from their parents or responsible ones to participate in the study and those with positive or non-determined IgA anti-transglutaminase (anti-tTG) antibody were followed-up for therapeutic investigation and guidance. This investigation was approved by the Ethics Committee in Research of the Nutrition School, Universidade Federal da Bahia. There was no conflict of interest.

Data collection happened in the school environment by qualified and previously trained professionals, during the period from July to December 2009.

Adolescents had their weights obtained through the Master® portable digital balance and heights through the portable stadiometer Leicester Height Measure®, and a maximum variation of 100 g and 0.5 cm was admitted for weight and height respectively. The body mass index was used to diagnose the nutritional status, adopting percentiles according to age and gender, proposed by the World Health Organization[18], categorized in thinness (< percentile 3), appropriate (≥ percentile 3 and ≤ percentile 85), overweight (> percentile 85 and ≤ percentile 97), and obesity (percentile > 97). Adolescents provided information regarding the presence of clinical manifestations (digestive and extra-digestive) that may be associated with CD, with the following answer alternatives: 1 = never, 2 = sometimes, 3 = rarely, 4 = frequently, and 5 = always, registered in a standard questionnaire. The answer "frequently or always" indicated the presence of clinical manifestation.

After a 12-hour fasting, 10 mL of the adolescents' blood were collected through the venous vein, in a proper environment in the school. Samples were duly conditioned and transported to the laboratory. The anti-human transglutaminase immunoglobulin A antibody (anti tTG-IgA) was adopted for this study as a specific serological test for CD screening, and determined by the enzyme-linked immune-sorbent assay (ELISA) using a diagnosis set of Orgentec Diagnostika GmbH, Mainz, Germany (GmbH). Results were expressed in arbitrary units (AU). Results higher than 10 AU were considered positive, and when they were lower than 7 AU, they were negative, according to the criteria recommended by the supplier of the diagnosis set.

Proportion was adopted for categorized data and mean (standard deviation) for the continuous variables in order to characterize the event in population. For data analysis, the statistical package SPSS (version 19.0) was used.

RESULTS

The mean age of students that composed the study was of 14.3 years old (standard deviation = 1.54 years old), and most of them was female (59.5%). The nutritional status was appropriate in 77.1% of the adolescents; thinness was identified in 7.7%; and overweight/obesity in 15.1%.

Two blood samples were lost due to hemolysis; therefore, the determination of the anti-tTG-IgA antibody was performed in 1,213 adolescents.

The anti-tTG-IgA antibody was positive in 6/1,213 (0.49%) adolescents. Values of anti-tTG-IgA antibody in the cut point considered undetermined (7 to 10 AU) were seen in five adolescents (0.41%), so they were classified with negative serology for CD. It was also found a case of CD confirmed in the childhood of a female adolescent, with negative anti-tTG-IgA antibody; however, since then treatment was being carried out with a gluten-free diet.

The characteristics of adolescents with positive anti-tTG-IgA are presented in the Chart 1. The appropriate nutritional status was seen in most adolescents (66.7%) that showed positive anti-tTG-IgA antibody; there was also an adolescent with thinness and another with overweight. The most reported digestive manifestation among these adolescents was constipation.

DISCUSSION

This is the first serological screening study for CD performed in adolescents from public schools in Salvador, Bahia, Brazil. This is the biggest capital of the Brazilian Northeast area, a fact that emphasizes even more the probabilistic profile of the sample. In the present study, 0.49% (6/1,213) seroprevalence was identified for CD, assessed by the anti-tTG-IgA antibody and one CD case confirmed by biopsy performed before the study.

Population-based studies that discuss about the CD seroprevalence characterization in Brazil are rare. Data of studies performed in specific populations identified that the positivity prevalence of the anti-TtG antibody varied from 0.28 to 1.76% in adolescents and adults from the South, Southeast and Northeast areas[12,17,19-21]. Seroprevalence through the anti-tTG IgA antibody of 3.80[22], 3.37[4] and 4.56%[14] were seen in children and adolescents of Pernambuco, percentages that are expressively higher than that seen (0.49%) in adolescents from Salvador. It is worth mentioning that studies done in Pernambuco were carried out in hospital and

Chart 1. Characteristics of the adolescents (11 to 17 years old) with positive anti-tTG-IgA serology identified at the public school network at Salvador, Bahia, Brazil, 2009.

Adolescent	Sex/Age	BMI (Percentile) Nutritional status	Main clinical findings
1	F/16	24.08 (84,5) Appropriate	Constipation, dyspepsia, pyrosis, anemia, stain in teeth, distension and abdominal pain
2	F/13	18.86 (45,8) Appropriate	Constipations, discomfort
3	M/12	17.04 (40.1) Appropriate	Absent
4	F/15	21.64 (68.8) Appropriate	Distension, irritability
5	F/15	15.67 (1.6) Thinness	Absent
6	M/12	20.82 (88.3) Overweight	Absent

BMI: body mass index (kg/m^2); F: female; M: male.

ambulatory populations, also one of them was performed with children and adolescents with low stature, which can be an atypical form of CD presentation[22].

In other regions worldwide, the CD seroprevalence, assessed by anti-tTG-IgA antibody, was registered in children and adolescents that were previously healthy, like in studies carried out in Libya (0.82%)[7], Belgium (0.86%)[23] and Italy (1.5%)[24]. In Saudi Arabia, a study with 1,167 healthy adolescents identified positivity of the anti-endomysial antibody in 2.2% of the subjects[25]. A seroprevalence that is considered excessively high (5.6%) was seen in children in the North of Africa, through the anti-endomysial antibody[6], which emphasizes that in different geographic areas, the CD serological prevalence is variable.

Recently, a study performed in the afro-descendent pediatric and adults populations in ten communities in Bahia, Sergipe and Piauí states, Brazilian Northeast side, registered absence of CD seroprevalence when the anti-endomysial antibody was assessed[26]. Thus, the authors suggested that different ancestral populations that populated different areas of Brazil may have influenced the variability found in the CD prevalence in the country[26].

It is believed that genetic, environmental variables, especially age when the child was exposed to gluten, as well as the quantity ingested in the diet, are factors related to the CD variable prevalence in different geographic areas[4,9,10]. Another condition that can explain the difference in CD seroprevalence identified in the studies is the selection of the sample. CD occurrence identified by studies that adopt ambulatory or hospital samplings can reflect the effect of selection, since these population-based segments can go to these services due to comorbidities associated with CD. On the other hand, adoption of a population-based sample can explain lower prevalence of CD. Variables like the screening method used, heterogeneity of the studied population and sensitivity of tests may also influence on the variability in the CD seroprevalence[9,10].

Seroprevalence studies are important for contributing to the early diagnosis of CD in asymptomatic subjects, choosing the ones that should be submitted to intestinal biopsy[4,5,7,11,16]. Until now, there is a general agreement that CD diagnosis can only be defined and the gluten-free diet can only be done after performance of digestive endoscopy with achievement of multiple duodenal biopsies that show alterations of mucosa inflammation and atrophy, compatible with CD[2,27,28]. This procedure is necessary since some subjects may present positive antibodies for CD, but do not satisfy criteria that define the presence of disease[27].

Several studies have showed and recommended the anti-tTG-IgA antibody as the best serological test available for CD screening, being useful to identify new CD patients with light symptoms, non-specific general complaints or extra-intestinal manifestations, as well as population and epidemiological tracking[2,3,7,12,23,29]. The determination of the anti-tTG-IgA antibody through ELISA is a reliable test with 90 to 99% sensitivity and a specificity of 94 to 100%[2,16,30,31]. It is worth mentioning that studies have showed that most subjects with positive anti-tTG antibody have CD confirmation through biopsy[2,16,31], and such test has a good correlation with the severity and extension of intestinal lesions found in the biopsy[2]. In Brazil, although the 2009 ministerial decree assures that this test has been performed by

the Brazilian National Unified Health System (SUS), until now such procedure has not been initiated in wide scale as it should[32].

Recently, it has been suggested the determination of the anti-tTG-IgA antibody though finger puncture[7,12] and through determination in the saliva[1,5] because they are simpler, more reliable and cheaper. It is believed these tests are useful for CD screening in a near future, especially in developing countries with limited conditions of diagnostic installations to perform immunologic assays[1,7,12]. Furthermore, quick tests can be used in ambulatories, medical clinics, and in big population-based studies[12].

The limitation of the present study was that the levels of IgA were not measured. Deficiency of IgA might be associated with CD, and under such conditions, the serologic test tends to yield false-negative results[8,15,23,30].

CONCLUSION

The present study identified a 0.4% CD seroprevalence among adolescents from public schools in Salvador, Bahia, Brazil. Thus, further investigations are necessary to confirm the prevalence of CD in other age ranges and in other risk population groups in Bahia and Brazil, especially because it is a disease associated with several frequent morbidities and complications of high prevalence, which deserve early diagnosis and appropriate therapeutic management.

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