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ABSTRACT

A strong association between celiac disease (CD) and dental enamel defects (DEDs) have been extensively reported, however, the nature of this relationship is still unclear. The aim of this study was to evaluate DEDs phenotype in CD individuals according to the time they were introduced to a gluten-free diet (GFD). Forty-five CD individuals were examined by a pediatric dentist. DEDs were classified according to the type of affected teeth. CD individuals were classified into two groups (with or without DEDs) and the differences between these groups were tested using chi-square or Fisher´s exact tests and *t*-test to compare differences between means. The Pearson coefficient test was used to evaluate the degree of the correlation between the age of GFD introduction and number of affected teeth. Individuals with MIH were introduced earlier to the GFD (p = 0.038). An association was also observed for molar DED (p = 0.013). In conclusion, our study suggested an association between a specific type of DED and the time that CD individuals were introduced to a GFD.

KEY WORDS: celiac disease, primary teeth, dental enamel, dental enamel hypoplasia, gluten-free diet

Assessing the proposed association between DED and gluten-free diet introduction in celiac children

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Introduction

Celiac disease (CD) is a chronic autoimmune enteropathy triggered by gluten exposition in genetically susceptible individuals.[1] CD worldwide prevalence is around 1% of general population and is one of the most frequent types of food intolerance.[1,2] However, only 10% to 15% of this population is correctly diagnosed since CD is often asymptomatic.[3] The treatment of CD is based on a gluten-free diet (GFD).[4–7]

The CD clinical signs and symptoms have a wide spectrum with different degrees, which can range from "classic" (e.g., chronic diarrhea, weight loss, pain and abdominal distension caused by villous atrophy of the small intestine)[4,7–10] to "atypical" that a lack of intestinal symptoms occurs (e.g., fatigue, iron deficiency anemia, dermatitis herpetiformis, short stature, and oral manifestations).[4,7,8]

Among oral manifestations, the two most common are recurrent aphthous stomatitis and dental enamel defects (DEDs).[4,5,7–9,11] DEDs are a common alteration in CD individuals and its prevalence ranges between 38% to over 80%.[7,8,12,13] DEDs tend to present a symmetrical phenotype in all four hemiarches,[5,8–11,14] in which the most commonly affected teeth are incisors and molars.[5,8–10]

Although a strong association between CD and DEDs has been widely reported,[4,5,7–10,14] the nature of this relationship is still largely unclear. There are some theories whether CD and DEDs share a common genetic background or if DEDs are caused by the CD nutritional deficiencies. Therefore, the aim of this study was to evaluate the presence of DEDs in CD individuals according to the time of the GFD introduction.

Material and methods

Ethical aspects

The Ethics Committee of the University of São Paulo of the School of Dentistry of Ribeirão Preto (Process 2010.1.1149.58) approved the research protocol.

Sample collection

Forty-five individuals in follow-up for CD in the Pediatric Gastroenterology Service of the University Hospital of

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Ribeirão Preto, Medical School of Ribeirão Preto, University of São Paulo.

The individual's clinical records were assessed in order to evaluate comorbidities, age of CD diagnosis/introduction of GFD.

Oral clinical examination and DEDs phenotype determination

A professional dental prophylaxis and oral examination were performed by a pediatric dentist (FKC) as previously described.[10] The professional was previously calibrated to assess DEDs. The kappa intraexaminator concordance index score was higher than 0.80.

DEDs were evaluated according to the Aine classification (grades I to IV)[5] using a clinical mirror under direct lighting. Dental fluorosis was excluded from DEDs phenotype. The DEDs classification is presented in Table 1.

None of the individuals had a history of dental trauma or apical periodontitis in the primary dentition.

Both specific and unspecific DEDs were recorded. Specific DEDs present a symmetric presentation involving the same teeth in the right and left hemiarches. DEDs were considered as nonspecific if a single tooth in one hemiarch (asymmetrical) was affected. Only permanent teeth were evaluated. All analyzed teeth were divided according to their groups (incisors, canines, premolars, and molars) and a specific group when molars and incisors were affected (MIH), according to the age of GFD introduction.

Statistical analysis

The CD individuals were classified into two groups according to the presence or absence of DEDs phenotype. The differences between DEDs group and without DEDs group were tested using chi-square or Fisher´s exact tests for the dichotomous variables. Odds ratio was used to calculate the relative risk among these groups. The *t*-test was used to compare the difference between the means. Kruskall–Wallis test followed by a Dunn's post hoc test was used to compare mean age of GFD introduction according to the Aine's classification of DED. The Pearson coefficient test was used to evaluate the degree of the correlation between the age of GFD introduction and group of affected teeth. The strength of the positive correlations was defined according the value of the "correlation coefficient" (r^2), such as:

- 1: perfect
- 0.7 to 0.9: strong
- 0.4 to 0.6: moderate
- 0.1 to 0.3: weak
- 0: no correlation.

All analysis was performed using Epi Info 3.5.2 and Graph Pad Prism 5.0a (Graph Pad Software Inc., San Diego, CA, USA). The established alpha was 5%.

Table 1. Classification of DEDs in D, based on Aine *et al.*[5]

Grade 0	No defects
Grade I	Defect in enamel color. Single or multiple cream-colored, yellow or brown opacities with clearly defined or diffused margins.
Grade II	Slight structural defects. Enamel surface rough, filled with horizontal grooves or shallow pits; light opacities and discolorations may be found.
Grade III	Evident structural defects. A part or the entire surface of enamel rough and filled with deep horizontal grooves that vary in width or have large vertical pits; large opacities of different colors or strong discolorations may appear in combination.
Grade IV	Severe structural defects. The shape of the tooth has changed: The tips of cusps are sharp-pointed and/or the incisal edges are unevenly thinned and rough; the thinning of the enamel material is easily detectable and the margins of the lesions are well defined; the lesion may be strongly discolored.

Results

Twenty-five (55.6%) individuals presented DEDs. DEDs did not have gender preference ($p = 0.297$) and was not associated with any comorbidities (OR = 1.0, 95% CI = 0.22-4.34; $p = 0.497$). In 92% of DEDs cases, the defects were specific. The characteristics distribution among the groups is presented in Table 2.

The age of CD diagnosis ranged from 24 to 180 months. The age of GFD introduction was 47.24 (±53.38) in DED individuals while in individuals without DEDs it was 49.60 (±37.53); there was no statistical difference between groups ($p = 0.86$; Figure 1). As for the groups of affected teeth, 33 of them were upper central incisor; 14 upper lateral incisor; 12 lower central incisor; 10 lower lateral incisor; 32 upper first molar; 33 lower first molar; 4 upper canines; 2 lower canines; 8 upper premolars; and 8 lower premolars.

Table 3 presents the mean age that the GFD was introduced according to the type of affected teeth. Individuals with MIH were introduced earlier to the GFD ($p = 0.038$). A strong association was observed for molar DEDs ($p = 0.013$) in the subgroup analysis.

There was no statistically significant difference between the age of the GFD introduction and the severity of DEDs according to Aine classification ($p = 0.0273$; Table 4).

The correlation test between age of the GFD introduction and the number of affected teeth by DEDs was weak (Figure 2; $r^2 = 0.150$; $p = 0.0195$).

Discussion

The literature has demonstrated that CD individuals have a lower salivary flow rate and buffering capacity[15] and DEDs.[5,8-11,16] Children with the first permanent molar affected by DEDs were associated with higher odds of dental caries experience.[17] Although the relationship between CD and DEDs has been well documented, the etiological aspects involved in their association are still

Table 2. Characteristics of the CD individuals.

Characteristics	Groups		*p*-value	OR (CI 95%)
	DED	Without DED		
Age mean (SD)	12.8 (5.6)	10.5 (5.5)	0.181	-
Gender n (%)				
Male	8 (32)	8 (40)	0.297	1.41 (0.41-4.83)
Female	17 (68)	12 (60)		
Comorbidities n (%)				
Yes	5 (20)	4 (20)	0.497	1.00 (0.22-4.34)
No	20 (80)	16 (80)		

Note: Comorbidities included: 2 cases of anemia, 1 case of gastroesophageal reflux disease, 1 case of malnutrition, and 6 cases of diabetes mellitus type I.

largely unknown. Our study aimed to unravel some aspects that could explain this association and found an interesting result.

Ameloblasts are highly specialized cells present only during dental development and they are responsible for the formation and mineralization of the enamel. Permanent enamel formation mainly occurs after postnatal period.[18] DEDs are related to the ameloblasts malfunction, because these cells are sensitive to many environmental and systemic alterations.[19,20] In fact, DEDs have been also associated with some serious and/or chronic illnesses[21,22] including CD.[4,5,7–11]

It is important to highlight the complex interconnection among immune system, microbiota, and environmental factors (including dietary food antigens and/or infection) involved in the CD etiology.[23] All these factors might be involved directly or indirectly with DEDs in CD individuals.

DEDs may be a result of the nutrients malabsorption, especially calcium, during the period of undetected CD.[5,7,8,11,14)] Therefore, the age at diagnosis and consequent GFD introduction could play an important role in enamel development.[8,13,16,24] However, if nutrient malabsorption was the main cause of DEDs, the time that the GFD started should influence the enamel defect phenotype; our results did not support this finding.

Another possible hypothesis, which was previously raised by some authors, is related to a specific immune response occasioned by HLA antigens with increase in the risk of development of DEDs.[4,5,8,14] It is based on the fact that in CD individuals, gluten-derived peptides are recognized by antigen-presenting cells, with T-cells response, in which deaminated gluten peptides are presented to T-cells leading to a subsequent release of proinflammatory cytokines,[23] this reaction would lead to a break of balance with consequent ameloblast damage. However, our study did not corroborate this hypothesis, since our correlation test did not find an association between GFD introduction and the severity of DEDs.

Our study was not able to find a positive association between age of GFD introduction and DEDs pattern, in which individuals diagnosed later, would have more DEDs. These are in agreement with Majorana *et al.* (2010)[8] and Trotta *et al.* (2013)[13] who did not find an association between age of diagnosis and enamel defects. Interestingly, our results demonstrated that MIH was more frequent in individuals that started the GFD earlier. This may be explained by the fact that when a patient is diagnosed younger, they have a more severe phenotype related to CD, such as chronic diarrhea, weight loss, pain, and abdominal distension,[4,7–10] which is probably associated

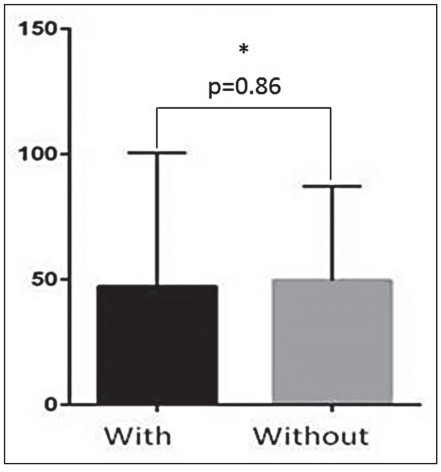

Figure 1. Mean age (in months) of GFD introduction according to the groups with and without DED.

Table 3. Mean age of GFD introduction according to the type of affected teeth.

Groups	Mean age in months (SD)				*p*-value
	No. of individuals	Yes	No. of individuals	No	
MIH	14	27.07 (±16.45)	31	57.87 (±52.46)	**0.038**
Incisors DEDs	21	48.95 (±36.46)	24	47.70 (±56.84	0.929
Canines DEDs	2	60.50 (±37.47)	43	47.72 (±47.18)	0.708
Premolars DEDs	2	25.50 (±12.02)	43	49.34 (±47.30)	0.484
Molars DEDs	16	25.68 (±15.78)	29	60.75 (±53.05)	**0.013**

Notes: Bold form indicates statistical significance difference.
The mean age of individuals without DEDs was 49.60 (±37.53) months.

Table 4. Association of age of the GFD introduction and grade of severity of DEDs according to Aine classification.

Grade according to Aine	No. of patients	Means age in months (SD)
0	20	49.60 (±37.53)
1	19	46.15 (±58.55)
2	5	57.80 (±35.60)
3	1	15.00
Note: There was no statistical difference between the groups (p = 0.0273).		

with the MIH defect, mainly molars. In fact, MIH has a genetic etiology as CD.[25] On the other hand, it is possible that our result is a false-positive association since we evaluated a small sample and used an α of 5% to avoid type I error.

If GFD introduction is not related with DEDs, it is possible that the nature of the association between DEDs and CD is through a common genetic background. In this scenario, genes involved in the etiology of the CD, such as HLA, might be also being expressed in the enamel development. About 90% to 95% of individuals with CD have a genetic alteration in *HLA-DQ2* gene and most of the remaining have an alteration in the *HLA-DQ8* gene.[6] A recent study also demonstrated a statistically significant correlation between oral manifestations and HLA expression.[14,26] However, so far, there are no studies with animal model that attempted to evaluate the expression of these genes during enamel development to confirm this hypothesis.

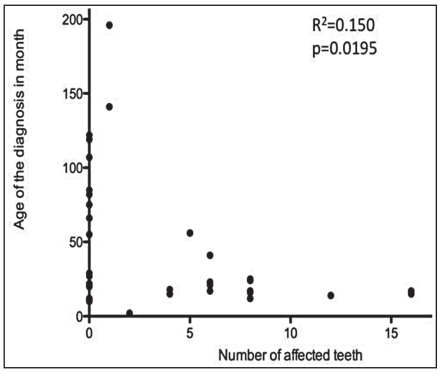

Figure 2. Correlation between age of the GFD introduction of CD and number of affected teeth by DDE.

More than 200 genes are expressed in dental development.[27] Therefore, it is also plausible that other genes involved in CD etiology are expressed in enamel development. A previous study performed a dense genotyping and identified multiple common new genetic variants associated with CD.[28] These genes, with a small effect in the CD trait, might also be involved in DEDs establishment.

Conclusion

Our study demonstrated an association between DED and the time that CD individuals were introduced to a GFD. Thus, further genetic studies are needed to investigate the nature of the relation between DEDs and CD.

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