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Implications of gluten exposure period, CD clinical forms, and HLA typing in the association between celiac disease and dental enamel defects in children. A case–control study

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Background. The association between coeliac disease (CD) and dental enamel defects (DED) is well known.
Aim. The aim of this study was to investigate the prevalence of DED in children with CD and to specifically find the association of DED and gluten exposure period, CD clinical forms, HLA class II haplotype.
Design. This study was designed as a matched case–control study: 250 children were enrolled (125 coeliac children – 79 female and 46 male, 7.2 ± 2.8 years and 125 healthy children). Data about age at CD diagnosis, CD clinical form, and HLA haplotype were recorded.
Results. Dental enamel defects were detected in 58 coeliac subjects (46.4%) against seven (5.6%) controls ($P < 0.005$). We found an association between DED and gluten exposure period, as among CD subjects the mean age at CD diagnosis was significantly ($P = 0.0004$) higher in the group with DED (3.41 ± 1.27) than without DED (1.26 ± 0.7). DED resulted more frequent (100%) in atypical and silent CD forms than in the typical one (30.93%). The presence of HLA DR 52-53 and DQ7antigens significantly increased the risk of DED ($P = 0.0017$) in coeliac children.
Conclusions. Our results confirmed a possible correlation between HLA antigens and DED.

Introduction

Coeliac disease (CD) is an immune-mediated disorder triggered by the ingestion of gluten in genetically susceptible subjects. The existence of an autoimmune diathesis underlying CD is suggested by the presence of nonorgan specific autoantibodies such as tissue anti-transglutaminase antibodies (tTG) and anti-endomysial antibodies (EMA) in the serum of CD subjects, and by the frequent association of CD with other autoimmune disorders such as insulin-dependent diabetes mellitus and thyroiditis[1,2]. The hallmark of CD is a gluten-sensitive enteropathy characterized by a variable degree of villous atrophy, hyperplasia of glandular crypts, and marked increase of intra-epithelial lymphocytes. The clinical spectrum is broad and includes *typical* forms usually presenting early in life with signs of intestinal malabsorption (chronic diarrhoea, weight loss, abdominal distension, developmental delay, etc.), *atypical* forms characterized by extra-intestinal manifestations, such as dermatitis herpetiformis, iron-deficiency anaemia, short stature, cryptogenic hepatitis, osteoporosis or osteopenia, ataxia, etc., and *silent* forms with mild and nonspecific gastrointestinal (GI) manifestations. Silent forms of CD may also occur and they are usually detected in first degree relatives of coeliac subjects who are subjected to serological screening by tTG or EMA[3–7].

Genetic, environmental, and immunological factors may play important roles in the pathogenesis of the disease. A significant association between CD and human leucocyte antigen (HLA) types was shown in many studies. CD was found to be mainly associated with HLA B8, HLA DQ2, and HLA DQ8[8].

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Oral manifestations, the most recurrent one is apthous stomatitis (RAS) and dental enamel defects (DED) are not uncommon in CD[9–11]. The association between CD and DED was described since the early 1990s when the implication of CD into the aetiology of the enamel mineralization anomalies was speculated[12,13]. These defects may range from discoloration to pitting, grooving, and total loss of enamel and are considered CD-specific when symmetrically and chronologically distributed.

The prevalence of CD-related enamel defects varies from 38% to 96% in different European countries[14–18] and several studies[19–21] consider this anomaly such as a potential clinical marker in the early identification of silent cases of CD.

The cause of DED in subjects with CD is still unknown. Several Authors suggest that hypocalcemia, due to malabsortion during the period of undetected disease, might provoke the enamel defects on the developing teeth. Therefore, the age at diagnosis and consequent gluten-free diet play an important role in DED onset[9,16,17,22,23].

Recent studies emphasize the rule of a specific immune response to gluten due to a genetic condition[15,23,24]. According to this hypothesis, many authors[9,16,17,25] found that specific HLA antigens significantly could increase the risk of dental lesions.

The aim of this study was to evaluate the prevalence of DED in CD children and to assess the association between DED and gluten exposure period and between DED and the CD clinical forms. Secondary aim of this study was to describe the distribution of HLA class II haplotypes in CD children with or without DED was investigated.

Materials and methods

Sample selection

This study was designed as a case–control study. Cases were identified as subjects affected by CD diagnosed at Gastroenterology Department of Pediatric Clinic of Brescia, and studied over a 18 months period (January 2007–July 2008).

One hundred and twenty-five children affected by CD (79 female and 46 male, mean age, and SD 7.2 ± 2.8 years), were enrolled in the study. A total of 125 healthy children matched to cases for age, sex, race, and area of residence were enrolled too. Not CD controls were selected among subjects referred to the Pediatric Dentistry Department of Dental Clinic at the University of Brescia, Italy.

Coeliac disease was diagnosed according to the criteria of the European Society of Paediatric Gastroenterlogy, Hepatology and Nutrition (ESPGHAN)[26]. For each subject, the CD clinical form (typical, atypical, *or* silent) was detected and recorded, taking into account to the clinical features, the histological and immunological abnormalities that had led to the diagnosis of CD.

In addition the age at CD diagnosis and the beginning of the gluten-free diet were also recorded. Medical and dental history for each child were also collected. Subjects with a medical history of infectious disease, dental trauma, treated with immunosuppressant drugs or chemotherapy or other medication with a known effect on the enamel mineralization were withdrawn from the study.

Methods

Dental examinations were performed by the same paediatric dental examiner (E.B). All subjects were placed in a dental chair. Their teeth were cleaned with an abrasive paste, washed, dried, and then observed in good artificial light, a small mirror, and a probe. All enamel defects observed, specific, and unspecific, were noted and photographed. Type and site of DED were recorded. DED were graded 0 to IV according to Aine's classification[12,14].

After obtaining consent from the children parents, HLA class II typing was carried out in all coeliac subjects and in the control group, using polymerase chain reaction-based DNA typing[27].

Data analysis

Initially, clinical condition parameters, were analysed univariately to describe the variables and distributions. Independent Student's *t*-test

between the two groups was calculated, and $P < 0.05$ was taken as significant level. To avoid the attenuating effect of unequal variability among groups on the value of *t*, a square-root transformation was performed when the response variable was a count. Secondly, a two-way table analysis was conducted to determine the crude odds ratio for the relationship between CD and controls.

Results

The gender and age distribution of the sample in the two groups is reported in Table 1. In CD group the onset of CD ranged from 8 months to 5 years. The mean age at the onset of CD was significantly ($P < 0.001$) higher in the CD subjects with DED (3.41 ± 1.27) respect to the CD subjects without DED (1.26 ± 0.7) (Table 2). Among the 125 subjects with CD, 97 (77.6%) had the typical clinical forms, 23 (18.4%) atypical forms, and five (4%) silent or subclinical forms of CD.

Prevalence of DED

In 58 of 125 CD children (46.4%), dental examination showed DED systematically distributed while in the control group only seven subjects (5.6%) had enamel defects ($P < 0.005$) (Fig. 1).

Dental enamel defects occurred in 261 teeth (69 primary and 192 permanent) of CD subjects and only in 10 permanent teeth of the control group. Enamel lesions in controls were significantly less frequent than in CD subjects ($P < 0.05$). Most of the CD children had grade I defects (47.5%), both in primary and permanent dentition. Grade I type enamel defects were the most commonly diagnosed also in the control group. DED were present in 30 (30.93%) of typical, 23 (100%) of atypical, and five (100%) of silent forms of CD (data not in tables) (Fig. 2).

In addition, 45/125 CD subjects (36%) showed black stain, a peculiar dental extrinsic discoloration, distributed along the third cervical line of the teeth (Fig. 3). Fifteen of 125 CD subjects showed black stain and DED contemporary. On the contrary, black stains were observed only in five of 125 (4%) of the control group.

Table 1. Distribution of the sample regarding age and gender.

Age (years)	Coeliac group (*n* = 125)		Control group (*n* = 125)	
	Females	Males	Females	Males
<2	6	4	6	4
3–5	14	8	18	13
6–12	51	42	50	38

Table 2. Subjects with coeliac disease (CD) with/without dental enamel defects (DED): number of subjects, mean age at dental examination and mean age at CD diagnosis.

	n (%)	Mean age	
		At dental examination Mean ± SD	At CD onset Mean ± SD
DED	58 (46.4)	8.25 ± 3.21	3.41 ± 1.27
No DED	67 (53.6)	6.40 ± 2.1	1.26 ± 0.7
		$P = 0.22$*	$P = 0.01$*

**t*-student.

HLA typing

The HLA DR and DQ haplotype in 125 children with CD and in the control group is displayed in Table 3. All coeliac subjects were positive to HLA DR 3/DQ 2 and DR 7/DQ 8 (100%), already associated to a significant increased risk of developing disease; in healthy subjects HLA DR 3/DQ 8 was completely absent and DR 7/DQ 2 was poorly represented (9.6%).

On the contrary, DR 1, DQ 4 e DR 4 antigens were present only in the control group. HLA DR 52–53 and DQ7 were statistically significative in CD DED+ subjects compared to CD DED−. In particular, HLA DR 52–53 resulted in eight of 58 CD+DED+ subjects and absent in CD+DED− ($P = 0.0017$) and DQ 7 was present in nine of 58 CD+DED+ subjects and in two of 67 CD+DED− ($P = 0.0136$). These antigens were not found in the control group.

Discussion

In this study, while few control subjects showed dental lesions, DED were observed in

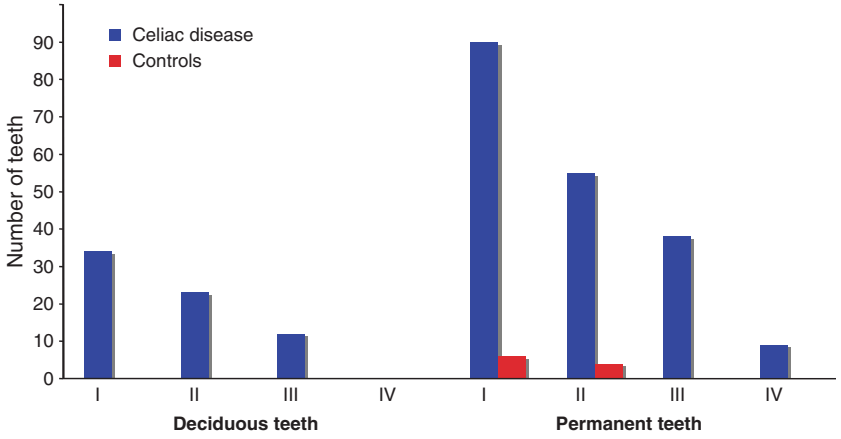

Fig. 1. Distribution of dental enamel defects (DED) in subjects with coeliac disease and in the control group.

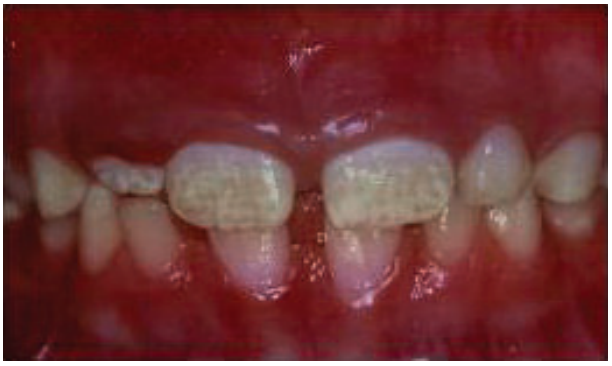

Fig. 2. Grade I type enamel defects.

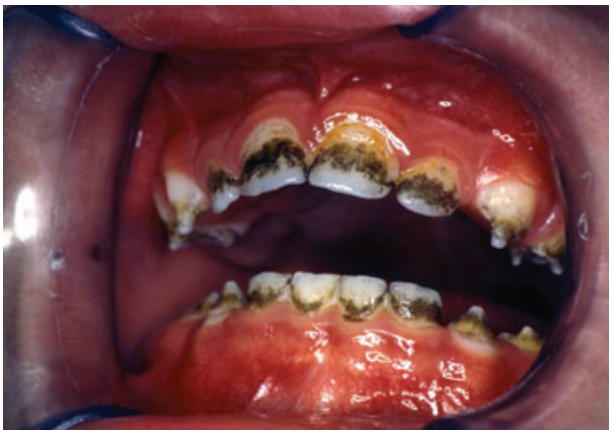

Fig. 3. A peculiar dental extrinsic discoloration, distributed along the third cervical line of the teeth.

about half of the examined sample. This percentage is higher than that described in different European countries[13,16] and in other Italian studies[9,25,28], but lower than that reported in Finnish[14] and the UK subjects[17] or in the Swedish coeliac group[11]. This variation in the prevalence of DED in coeliac subjects, among different geographical areas, may depend more on the polymorphism of the diseases and linked to complex process to diagnose the disease. However, this wide difference in prevalence may justify the scientific debate about the existence of an association between CD and DED and its nature. A potential role could be played by the metabolism of calcium on the developing dentitions and consequently the age of diagnosis of CD is quite important. It is described that early diagnosis and consequent gluten-free diet could prevent, or reduce, enamel lesions[9,15,21,28]. A quite high statistical significant association was described between the time of gluten challenge (according to ESPGHAN diagnostic criteria) and DED development[13]. In this study, an association between DED and the gluten exposure period

Table 3. Distribution HLA (DR and DQ) in the different groups of subjects with coeliac disease with dental enamel defect (CD+ DED+), without dental enamel defect (CD+ DED) and in the control group.

	CD group (*n* = 125)		
Antigens	**DED (*n* = 58) *n* (%)**	**Not DED (*n* = 67) *n* (%)**	**Controls *n* (%)**
DR1	–	–	108 (86.4)
DR3	30 (51.7)	32 (47.8)	–
DR4	–	–	120 (96.0)
DR5	10 (17.2)	8 (11.9)	3 (2.4)
DR7	28 (48.3)	35 (52.2)	12 (9.6)
DR52-53	8 (13.8)	–	–
DQ2	30 (51.7)	32 (47.8)	12 (9.6)
DQ3	–	5 (7.5)	120 (96.0)
DQ4	–	–	108 (86.4)
DQ7	9 (15.5)	2 (3.0)	–
DQ8	28 (48.3)	35 (52.2)	–

was observed; in coeliac subjects the mean age at diagnosis was significantly higher in the group with DED than in the group without DED. These results are similar to those reported by another Italian study[28], where it was concluded that the age at diagnosis plays a role in determining the number of affected teeth. While, in another study[9], the mean age at diagnosis did not statistically differ between subjects with and without enamel defects. According to this study, the presence of an association between DED and the gluten exposure period seems also to be confirmed by the high percentage of primary teeth involved. In literature there are few studies regarding DED of primary teeth in CD children[17] but, as the crown mineralization of the primary teeth begins 4–5 months before and ends 6–12 months after birth, it is likely that also DED of primary teeth are related to gluten, which is usually introduced in the diet after the 5th month of life.

In addition, the presence of DED, is significantly more common in atypical and silent forms of CD than in the typical one, in this study, could be explained by the late and troubled diagnosis of the disease due to the poor and specific clinical symptoms. These results may be related to the early gluten-free diet set up in the typical form that may protect from DED manifestation.

A role in DED developing in CD subjects could be played also by genetic factors. Maki *et al.*[24] suggested a possible association between HLA DR3 antigen and DED in coeliac subjects. These findings were demonstrated also by Mariani *et al.*[25] that observed how the presence of HLA DR 3 antigens increased the risk for DED while HLA DR 5,7 seemed to protect from the occurrence of dental changes.

These results confirmed the association of HLA DR 3 with CD but no association with DED. The same result for HLA DR 5 and 7 were observed, statistically significant in CD subjects but not relevant for the presence of DED. On the other hand, in coeliac subjects, the presence of HLA DQ7 and DR 52–53 antigens was significantly associated with dental lesions, thereby suggesting that they carry an increased risk of DED.

The association between HLA DR3/DR7 and CD is explained by the linkage disequilibrium of these alleles with DQ allele, even if difference in HLA distribution may be associated with genetic heterogeneity and geographical region[8].

The differences in the prevalence of DED reported in studies from different countries may be partially justified by the different distribution of HLA antigens as HLA class II antigens are represented differently in different populations, in particular in northen European subjects[28] frequency of HLA DR3 is 95%, whereas in southern Europe, HLA antigens DR3 and DR7 are the most common HLAs[17,24,29]. Possibly, the origin of DED in CD children is due to multifactorial events and further studies are needed to investigate other determinants.

Besides DED, in this study the black stain, a previously unreported abnormality of dental enamel, was found in subjects with CD. The presence of black pigmentations of the hypoplasic teeth was documented also by a recent study[21,30], and seems to have a confirmatory meaning of the systemic unbalance which influence the oral microflora of these subjects.

What this paper adds

- This paper try to provide a possible explanation of DED origin in CD children, relating DED to different determinants, i.e., gluten exposure period, CD clinical forms, HLA class II haplotype.

Why this paper is important to paediatric dentist

- In daily routines, the detection of specific dental enamel defects in children might help in early diagnosing CD, overall silent forms.

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