Enamel hypoplasia in coeliac children: a potential clinical marker of early diagnosis

M. BOSSÙ, A. BARTOLI, G. ORSINI*, E. LUPPINO*, A. POLIMENI

ABSTRACT. Aim To assess at the scanning electron microscope (SEM) the structural aspects of enamel hypoplasia (EH) in coeliac disease (CD) with the aim to investigate our hypothesis of a possible significant difference between structural characteristics of EH in CD affected patients and EH of non-coeliac patients. If the presence of specific features of the EH associated with CD were demonstrated, these findings would represent for the dentist early non-invasive clinical markers of diagnosis of CD in case of suspected disease. Methods We analysed at SEM two samples of enamel fragments from hypoplastic teeth, both deciduous and permanent, harvested from 10 coeliac children (18 permanent teeth, 6 deciduous teeth; study group) and 10 non-coeliac children (16 permanent teeth, 4 deciduous teeth; control group) treated for dental caries, dental extractions for extensive caries lesions or deciduous teeth exfoliation. Results Significant structural differences were noted between EH of non-coeliac patients and same dental lesion in the group with CD. In the study group, EH defects were predominantly located on the central and lateral incisors, upper and lower, both deciduous and permanent, and on the first permanent molars, and were always symmetrical. EH of permanent teeth of CD affected patients was characterised by prisms more irregularly distributed with irregular margins and less interprismatic substance than observed in non-coeliac EH. The deciduous teeth of the study group showed shorter enamel prisms with a non-parallel direction up to convergence and less interprismatic substance than observed in the control group. Conclusion This morphological analysis at SEM of the hypoplastic enamel defects of a group of coeliac children, the first published in literature, demonstrates that the EH of deciduous and permanent teeth in CD is highly hypomineralised with shorter prisms, more irregularly distributed and less interprismatic substance than observed in the non-coeliac EH. More data are needed to validate the significance of our observations with the aim to assess if this simple, non-invasive microscopic analysis can be considered effective for the early identification of silent cases of CD that otherwise would not be diagnosed in the paediatric age.

KEYWORDS: Children, Coeliac (celiac) disease, Enamel hypoplasia, Early diagnosis, Scanning Electron Microscope.

Introduction

Coeliac disease (CD) is a permanent disease characterised by severe cell-mediated immune lesions of the intestinal mucosa of patients with genetically determined intolerance to gluten, which is a protein contained within wheat, rye, barley, and possibly oats. The CD is a pediatric disease, diagnosed very early between the 9th and 12th month of life, after the introduction of the gluten in the diet, with symptoms of intestinal enteropathy. The existence of silent clinical patterns of the disease, where the gluten of the diet causes a slow and inexorable intestinal damage, in the absence of clinically evident gastrointestinal symptoms, demonstrates the importance of early diagnosis for the health of these young patients [Ciclitira et al., 2005]. In Europe, CD affects one in 100 people, whereas it is very rare or absent among blacks and Asians [Collin et al., 1997]. Large differences in incidence between geographic areas of Northern and Southern Europe have been documented: in the South the typical and early clinical patterns of the disease are predominant, whereas in the North latent forms of CD are more frequent; in Sweden diet and genetic predisposition have favoured
the selection of a population at an increased risk of either types of CD [Collin et al., 1997]. These differences in the prevalence of CD depend more on the polymorphism of the CD and its difficult diagnosis than on true differences of prevalence based on determinantal environmental factors; indeed, the slight higher prevalence of CD in the South of Europe, until few years ago, depended on the easy diagnosis of the typical forms of CD, as opposed to the silent and latent patterns more frequent in the North. Today, the use of serologic markers, such as anti-gliadin antibodies (AGA), anti-endomysial antibodies (EMA), anti-jejunum antibodies, and more recently anti-transglutaminase antibodies (anti-tTG) [Dicke and Hughes, 2000; Fasano, 1999; Picarelli et al., 1996; Russo et al., 1999] allow for an increased diagnosis of clinical patterns of latent and silent CD, which represent the hidden part of the true prevalence of CD (iceberg theory) [Corazza et al., 1997]. The pathogenic development of CD requires two factors: one being genetic, related to the human leukocyte antigenic system (HLS) of identification of the foreign antigens, where Class II- DQ2- and DR3-HLA molecules play the role of presenting the gluten-antigens to the T-lymphocytes within the intestinal mucosa, thus enabling for an immune response to this antigenic complex. The other factor is related to the diet and the presence of gliadin, which is the soluble glycoprotein extracted from gluten, where the amino acid sequence 31-43 is the active antigen fraction against which the cell-mediated immune system reacts releasing AGA and increasing the production of molecules HLA-DQ2 and HLA-DR3 [Picarelli et al., 1999].

CD manifests with the typical clinical pattern of the dystrophic child. After few ingestions of the gluten, the rising gastrointestinal symptoms are chronic diarrhoea, abdominal pain and distension, and weight loss. Although the target organ of the CD is the jejunal mucosa, there are other manifestations (dermatological, neurological and dental), primarily related to malabsorption, poor feeding, and immunologic disorders [Abenavoli et al., 2006; Martin et al., 2006; Rasmusson and Eriksson, 2001; Aine et al., 1990]. These non-gastrointestinal manifestations have been more frequently described in young adults as characteristic of the latent and silent forms of CD and their intensity is related to the patient’s age [Ferguson et al., 1993]: growth retardation with short stature, iron-related anaemia (not solved by oral ingestion of iron), dermatitis herpetiformis, irriability, dental enamel hypoplasia (EH) and opacity, recurrent and persistent oral ulcerations, arthritis, hepatitis, thyroid dysfunctions, diabetes, nail and hair fragility [Bucci et al., 2006; Majorana et al., 1992; Aine et al., 1992]. The oral manifestations are related to IgA deficiency (mucosal-immune system), delayed dental eruption, and the defects in the enamel differentiation of deciduous and permanent teeth [Mariani et al., 1994; Aine, 1994]. The enamel defects associated with the CD have been explained by two mechanisms: lesions due to malabsorption and resulting hypocalcemia [Smith and Miller, 1979; Aine, 1986], and enamel lesions rising form the autoimmune response against ameloblasts [Maki et al., 1991]. The enamel defects can manifest precociously in the 3rd-6th month of life with little horizontal bands, localised pits and opacities, characteristically bilateral and symmetric. These defects are localised in areas of the crown in development, affecting in chronological order the first molars, canines, and second molars of the deciduous teeth, and first molar, central incisors, canines, lateral incisors, first and second premolars of the permanent dentition. In table 1 are summarised the bilateral and symmetric enamel lesions systematically described for the first time by Aine [Aine, 1986]. Other studies aimed to identify associations between various oral lesions and dental enamel defects as possible early diagnostic markers of CD [Aine, 1996; Majorana et al., 1992].

The aim of this work was to evaluate if EH, which is the most frequently observed dental lesion associated with CD, shows at SEM specific characteristics in comparison with EH of non-coeliac children. Our study is based on the hypothesis of a possible specificity of the structural characteristics of the EH in children with CD compared with the EH of other patients. If this hypothesis were confirmed by evidence-based microscopic studies, this oral manifestation, in the presence of other symptoms of the disease, would represent for the paediatric dentist an early non-invasive clinical marker to intercept CD.

Materials and methods

Medical history. Information related to diagnosis of coeliac disease (according to the 1970’s criteria of the European Society of Paediatric Gastroenterology and Nutrition), use of fluoride supplements, such as drops, tablets and toothpaste, and the age of elimination of the gluten from the diet were collected by a structured questionnaire proposed to parents.

Study population. At the Paediatric Dental Department, “La Sapienza” University of Rome, two paediatric populations were selected with EH of deciduous and permanent teeth as common feature: 10 children affected by CD (age: 2-9 years) and 10 non-
coeliac children (age: 3-11 years). The criterion used to match children were: race (Caucasian) and age, which are two parameters that can influence the degree of mineralisation of the crown, age of start using fluoride supplements that could have altered structure and colour of the hypoplastic enamel, and age of elimination of gluten from the diet, because at the time of gluten introduction IgE could enter the maturing enamel and inhibit full maturation, thus giving rise to enamel defects and opacities.

**Enamel defects.** After having cleaned and dried the teeth, the enamel defects were diagnosed and classified by one skilled paediatric dental examiner according to the criteria described by Aine [Aine, 1986] and reported in table 1. We selected only “gross” structural defects (grades II, III and IV of EH), preferably associated to dental caries, on deciduous and permanent teeth. We never considered the teeth showing opacities alone because of the therapeutic goal to restore the gross structural enamel lesions, when feasible, while harvesting the surrounding hypoplastic enamel. The enamel samples, harvested during restorative procedures for dental caries or dental extractions for extensive carious lesions or exfoliating teeth, were 18 from permanent teeth and 6 from deciduous teeth in the CD group; 16 from permanent teeth and 4 from deciduous teeth in the non-coeliac group. The enamel fragments were collected into plastic tubes containing 10% buffered formalin. Then, in collaboration with the Department of Dental Sciences of the University of Chieti (Italy), the two enamel sample groups were analysed at SEM by one examiner to compare the characteristics of the structural hypoplastic enamel defects with the aim to assess if there were some features that could be specifically referred to CD (while being absent or less “intense” in EH of non-coeliac patients). Each sample was dehydrated in ascending alcohol series, etched with 37% orthophosphoric acid for 30 seconds to display the enamel prisms, then mounted on stub and finally metal-treated (Emitech K 550, Emitech Ltd, Ashford, Kent, UK) to be observed at SEM (LEO 435 Vp, LEO Electron Microscopy Ltd, Cambridge, UK).

**Statistical analysis.** No statistical analysis was performed on the qualitative data because of the scarce homogeneity of the structural enamel aspects and different “donor” crown areas.

### Table 1 - Characteristics and grading of dental enamel defects in coeliac disease.

<table>
<thead>
<tr>
<th>Grading</th>
<th>Dental enamel defects</th>
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<tbody>
<tr>
<td>0</td>
<td>No defects</td>
</tr>
<tr>
<td>I</td>
<td>Defects of colour: single or multiple opacities, cream-colored, yellow or brown, with well defined or diffused margins; one portion of the crown is interested.</td>
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<tr>
<td>II</td>
<td>Mild structural defects: rough enamel surface with horizontal grooves and superficial pits; possible mild opacities and discolorations; one portion or the whole crown can be interested.</td>
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<tr>
<td>III</td>
<td>Evident structural defects: one portion or the whole crown is rough or is covered with grooves with varying degrees of deepness and width or there are large and deep pits; there can be opacities of different colors and discolorations.</td>
</tr>
<tr>
<td>IV</td>
<td>Severe structural defects: the shape of the tooth is changed with sharp cusps and incisive margins that are irregularly thin and rough; the thinning of the enamel is generalised and the edge of the lesions is well defined; the lesions can be hardly discoloured.</td>
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**Results**

**Fluoride use.** No pre-natal use of fluoride supplements was reported by the children’s mothers, whereas all children had received fluoride supplements as tablets since diagnosis of CD and in half of the cases children had received preventive sealing of their molars.

**Enamel defects.** Grades II, III and IV of EH were observed in children with and without CD. At SEM there were significant differences in some of the structural elements of the EH between the CD group (N=24) and the non-coeliac group (n=20). The children affected by CD showed a preferential symmetrical distribution of EH on the upper and lower central and lateral incisors, both deciduous and permanent, and on the first permanent molars. From a microscopic point of view, in the non-coeliac group the EH of permanent teeth was characterised by a regular distribution of the enamel prisms that were clearly evident and separated by a different quantity of interprismatic substance in relation with the grade of the hypoplastic defects (Fig. 1, 2). In the permanent teeth of the coeliac group, in the porous areas of the EH, the crystals showed a normal direction and morphology of the base with spotted margins and evident interprismatic substance; in the hypoplastic areas adjacent to the normal enamel, the prisms were
grouped and irregularly shaped and, in the middle of
the hypoplastic defect, were separated by abundant
interprismatic matrix; the margin between the normal
and the hypoplastic enamel was marked by the
direction of the prisms.

In general, the EH of the coeliac teeth showed more
irregularly distributed prisms, less adherent, with
irregular margins and dispersed into scarce
interprismatic matrix (Fig. 3-6). In the deciduous
teeth of the coeliac patients, the direction of the
prisms was unparallel up to converge, they were
shorter than that of the deciduous teeth of the non-
coeliac patients and the interprismatic matrix was
less represented (Fig. 7-10). Black pigmentation
was evident both on the permanent and deciduous
teeth of the coeliac patients.

FIG. 1, 2 - Enamel hypoplasia of a first permanent molar of non-coeliac patient: regular distribution of the prisms and well
evident interprismatic substance.

FIG. 3 - Enamel hypoplasia of a first permanent molar of
a coeliac patient: irregular distribution of the prisms and
scarce interprismatic substance.

FIG. 4 - Enamel hypoplasia of a first permanent molar of
a coeliac patient: scarce definition of margins of the
prisms.

FIG. 5 - Enamel hypoplasia of a first permanent molar of
a coeliac patient: scarce definition of the interprismatic
substance (higher magnification).

FIG. 6 - Enamel hypoplasia of a first permanent molar of
a coeliac patient: scarce definition of the margins of the
prisms (higher magnification).
Discussion

Our experimental report is the first published in PubMed, as to our knowledge, on the structural characterisation at SEM of the EH of deciduous and permanent teeth of coeliac children.

The analyses at SEM of EH in coeliac patients employed in previous studies regard the prevalence of such defects [Farmakis et al., 2005; Priovolou et al., 2004]. In the study of Farmakis, the enamel defects were diagnosed by the DDE Index (1989), whereas in our study the criteria of diagnosis and classification of enamel defects were those described for the first time by Aine (1986) and used in other studies on enamel defects [Mariani et al., 1994; Aguirre et al., 1997; Ventura and Martelossi, 1997]. We selected enamel defects type II, III and IV while refusing to harvest crown enamel areas showing opacities alone because of the possibility that this lesions can remineralise.

According to some authors [Andersson-Wenckert et al., 1984; Rasmusson et al., 2001] CD does not significantly impact the presence of enamel defects compared with the normal population. Although in the present study, we always reported almost two dental lesions type II, III or IV in each child of the celiac group. The clinical diagnosis of dental lesions in CD affected children is always advised in order to have significant statistical data to demonstrate the specificity of some aspects of EH in such patients and thus having a new diagnostic sign for the early diagnosis of the disease. This simple, non-invasive diagnostic method has the potential to widen the diagnostic armamentarium for the early identification of silent cases of CD in children presenting enamel lesions that otherwise would not be diagnosed. Harvesting of the enamel can be easily performed by a dental practitioner using common instruments (air-spray cooled diamond burs) during the treatment of dental caries or on extracted hypoplastic teeth.

Dental enamel develops in two phases: the ameloblasts at first deposit the matrix of the hydroxyapatite crystals (secretion and architectural formation) with a tooth-specific spatial architecture and direction that corresponds to the normal enamel structure and, after, the crystals undergo mineralisation (maturation or mineralisation) with contemporary elongation and enlargement of their size. The alterations of shape and size of the enamel prisms observed at SEM in the teeth of our coeliac
patients correspond to a damage occurred during early phases of development of the dental crown during the first year of life. Different aetiologic factors have been related to these defects, such as the scarce supply of oxygen to ameloblasts, dioxin in the maternal milk, respiratory diseases, or other environmental conditions [Aguirre et al., 1997]. Through mass ion-spectrometry and x-ray microanalysis, the hypomineralised areas of EH have been shown to contain an increased amount of C and a lower amount of calcium and phosphorus, compared with the normal enamel [Jalevik et al., 2005]. In literature, some genetic, immune, and systemic factors able to impair the amelogenesis have been also described, such as gluten-mediated IgE or albumin, capable to disturb the maturation process and cause the enamel structural defects and opacity [Farmakis et al., 2005]. Our coeliac population was free of the possible determinant factors reported above, as referred by their mothers in the interview.

The time period elapsed between the initiation and diagnosis of the CD and the subject’s age may have significant effect on the severity of the enamel defects because of the possible use of fluoride supplements as tablets or toothpastes, which can affect the presence and severity of the enamel defects on permanent teeth. Indeed, although the deciduous dentition can be affected only if the malabsorption interests the mother, and thus also the fetus’s primary dentition, the permanent dentition is affected by the time the CD has well established in the child and therefore all permanent teeth are interested by the altered amelogenesis (except for the cusps of the first permanent molars which develop and calcify in utero). As reported in the Privolou’s study [2004], all the enamel defects observed in the CD group were of grade I, whereas in the corresponding group of the Aine’s study [1986] most defects were of grade II, because there was a difference between the two studies in the age of diagnosis and treatment of the patients with subsequent impact on the enamel mineralisation process.

In view of the considerations above, in our study we intended to find significant signs of impairment both of the architectural spatial distribution and of maturation of the enamel prisms in the primary dentition, while searching for common signs of altered spatial distribution of the enamel prisms in the permanent dentition, being the mineralisation pattern “biased” by the fluoride supplements intake.

Our observations at SEM of the presence of more severe alterations of shape and length of the crystals (shorter prisms with irregular margins), prismatic spatial distribution and amount of interprismatic substance in slides of EH of our coeliac patients, as a whole, demonstrate that these enamel defects are less mineralised than the control group and may have occurred both during the early formation and mineralisation of the crown. Differences in the symmetrical distribution of the enamel defects were significant between CD and control group. The black pigmentations observed on the hypoplastic teeth of the coeliac patients and absent on the hypoplastic teeth of the non-coeliac ones confirm the observation that the dental lesions in CD may have distinguishable aspects because of altered systemic conditions. Indeed, the black pigmentations may possibly be referred to chromogenic microorganisms (normally absent in the oral microflora) infiltrating the enamel defects which demonstrate an alteration of the oral microflora of these patients.

Conclusion

Our findings demonstrate that the hypoplastic enamel of coeliac patients is weaker than that of the non-coeliac patients because of shorter prisms, more irregularly distributed, and less interprismatic substance. Some microscopic findings were “more evident” in the coeliac group, such as very short enamel prisms and the impairment of the prismatic margins. In the coeliac group, we report the “specific” bilateral and symmetrical localisation of the enamel defects, and their predominant location on the deciduous and permanent upper and lower incisors, and on the first permanent molars. The presence of black pigmentations on the hypoplastic teeth of our group of CD affected patients have a confirmatory meaning of the systemic unbalance which influences the oral microflora. The results obtained from the little sample analysed in this study need to be confirmed by other data on the appreciable structural differences of the enamel defects in order to be validated.

References


