Deciduous teeth structure changes in congenital heart disease: Ultrastructure and microanalysis

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(Received: April 4, 2014; Revised manuscript received: May 26, 2014; Accepted: July 12, 2014)

Abstract: Objectives: Oral manifestations recorded for congenital heart disease (CHD) patients include teeth hypoplasia and high caries incidence. These observations suggested that the enamel and dentin of the teeth may be altered, increasing the risk for caries incidence. This study was designed to investigate the effect of CHD on the ultrastructure and composition of deciduous sound teeth. Methods: Thirty sound exfoliated human deciduous incisor teeth were selected for this study. They were divided into three groups, Group I (control) from healthy children (n = 6), Group II from acyanotic CHD children (n = 12) and Group III from cyanotic CHD children (n = 12). Each tooth was longitudinally sectioned, providing enough specimens for ultrastructure and chemical analysis using ESEM/EDAX. The results of ESEM/EDAX and dentin image analysis were statistically analyzed using one-way ANOVA test followed by Tukey’s test. Results: Enamel of groups II and III showed increased dissolution and irregular orientation of enamel prisms. Orifices of dentinal tubules demonstrated widening and irregular outlines, also lateral branching increased markedly. Image analysis of dentin ESEM photomicrographs showed a highly significant increase in surface area of dentinal tubules. Decrease in calcium (Ca) and phosphorus (P) levels was statistically significant (P < 0.05). Conclusions: CHDs affect the structure and chemical composition of deciduous teeth.

Keywords: congenital heart disease, deciduous teeth, ESEM/EDAX

Introduction

Congenital heart diseases (CHDs) are abnormalities in the cardiocirculatory structure or function due to abnormal heart development during fetal life [1, 2]. Among birth defects, CHDs are the most common type [3], representing approximately 0.4% to 1% of all live births [1]. A recent study reported that the prevalence of CHD among Egyptian school children accounted for 1.01/1000 [4]. CHDs are classified into: cyanotic CHDs (cause blue discoloration due to relative lack of oxygen) and acyanotic CHDs [2].

Changes in bone density and bone age delay in CHD children have been reported. Oral manifestations recorded for patients with CHD were Cyanosis, pale tissues and cleft palate and lip [5]. Other clinical findings associated with CHD include delayed teeth eruption, teeth hypoplasia [6, 7] and high caries incidence [8].

We hypothesized that enamel and dentin mineral content and structure in CHD patients may be altered, providing an increased risk for caries incidence in individuals who are prone to infective endocarditis. Furthermore, by proving these changes in enamel and dentin, protection of newly erupted teeth of CHD patients will be recommended, also further investigation regarding restorative techniques and materials used for the teeth of CHD patients will be required.

The integrity of enamel and dentin structures is crucial to the vitality and function of the tooth. The ultrastructure and composition of healthy enamel and dentin account for their ability to resist the effects of cariologic bacteria and other destructive factors in the oral environment. Literature review reported that CHD may lead to changes regarding oral health [9]. However, the possibility that dental hard structures of CHD children may be compromised was not fully explored.
This study was designed to find whether CHD affects the structures and/or chemical composition of enamel and dentin of deciduous incisors thus, increasing the risk for caries incidence and restoration failure.

Materials and Methods

Thirty exfoliated human deciduous incisor teeth were selected for this study; they were free of caries and any other visible defects. The teeth were collected according to a protocol approved by the Ethical Committee of Mansoura University, Mansoura, Egypt. All the teeth were cleaned thoroughly, (stored in sodium azide solution 0.002%) at 4 °C and used within 1 month.

For all patients, information on medical history was retrieved. They were subjected to full clinical and echocardiographic examination by an experienced pediatric cardiologist. Pulse oximetry was obtained for all cyanotic patients. Also none of the CHD children had syndromes or diseases affecting other body organs.

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**Fig. 1.** Representative ESEM photomicrographs (x2000) of enamel. At (A) group I shows type II etching pattern and enamel prisms exhibit regular orientation. (B) Group II showing enamel with type II etching pattern. Dissolution of some prism heads is apparent (arrows). Enamel prisms show slightly irregular orientation (dashed arrows). Many prism heads exhibit non-uniform outlines. (C) Group II showing enamel with type II etching pattern. Dissolution is increased and non-homogeneous (arrows). (D) Group II showing type III etching pattern. Dissolution is marked, some areas show dissolution of both prisms and interprismatic substance (encircled). (E) Group III showing type II etching pattern. The dissolution of interprismatic substance increased (arrows). There is change in prism orientation (dashed arrows). (F) Group III showing increased dissolution of interprismatic substance (arrows)
The collected teeth were divided into three groups, Group I (control) from healthy children ($n = 6$), Group II from acyanotic CHD children ($n = 12$) and Group III from cyanotic CHD children ($n = 12$), one tooth from each child. Each tooth was longitudinally sectioned, providing enough specimens for ultrastructural analysis using Environmental scanning electron microscope (ESEM) and chemical analysis using Energy dispersive analytical X-ray (ESEM/EDAX).

ESEM/EDAX was used to measure Ca and P levels in enamel and dentin of the tooth specimens.

Before ESEM analysis, enamel and dentin surfaces were polished and then etched. Enamel was etched for 15 seconds using 35% phosphoric acid gel (3M ESPE, St. Paul, MN, USA) while dentin was etched for 20 seconds using 37% phosphoric acid gel (Alpha-etch 37). After etching, the surfaces were rinsed with water spray for 30 seconds and dried for 30 seconds. The entire surfaces of treated enamel and dentin were examined under ESEM, however, only photomicrographs of representative surface areas were taken.

Image analysis of dentin ESEM photomicrographs was carried out to determine the surface area occupied by dentinal tubules. The images were analyzed on Intel® Core 13® based computer using Video Test Morphology® software (Russia) with a specific built-in routine for automated % area calculation and object counting.

Statistical analysis was performed for assessing the impact of CHD on the % area of dentinal tubules, also for evaluating the effect of cyanotic and acyanotic CHD on mineral contents of deciduous teeth enamel and dentin. Comparisons were carried out by analysis of variance (ANOVA) followed by Tukey’s post-hoc test. Significance was considered when $P$ value < 0.05.

Results

Micro-morphological observations of the enamel surfaces with ESEM

Group I (control), enamel showed normal prisms and interprismatic substance, type II etching pattern was evident. The enamel prisms exhibited regular orientation (Fig. 1A).

Group II (acyanotic), showed type II etching pattern with increased and non-homogeneous...
enamel dissolution. Also the number of dissolved prism heads increased (Fig. 1B). Other prism heads manifested non-uniform outlines and showed irregular orientation (Fig. 1C). Some specimens showed type III etching pattern. There were areas that showed dissolution of both prisms and interprismatic substance (Fig. 1D). Group III (cyanotic), demonstrated increased dissolution of interprismatic substance (Fig. 1E, 1F) and irregular orientation of enamel prisms (Fig. 1F).

Micro-morphological observations of the dentin specimens with ESEM

Group I (control), orifices of dentinal tubules showed normal circular outlines. The rest of the structures were normal (Fig. 2A). Group II (acyanotic group), tubule density (count/area) increased. The dentinal tubules showed irregular outlines and demonstrated widening to the extent of becoming connected with neighboring lateral branches (Fig. 2B). Peritubular dentin appeared thinner and was not easily discerned. Lateral branches of dentinal tubules markedly increased (Fig. 2B, 2C). Group III (cyanotic group), dentinal tubule orifices outlines were highly irregular in shape. There was a wide range of dentinal tubules sizes, some were narrower than the control and others were wider even than those of the acyanotic group. Intertubular dentin exhibited a granular appearance. Peritubular dentin appeared thinner. Lateral branching increased (Fig. 2D).

The results showed differences in the calculated surface area between the subgroups. Dentinal tubules and their branches showed a highly significant increase in surface area in group II and group III when compared with group I (Table I).

Chemical analysis of dentin and enamel surfaces with energy dispersive analytical X-ray (ESEM/EDAX)

Mineral analysis revealed changes in the levels of Ca and P of groups II and III. The levels of Ca and P decreased in

| Table I | Statistical analysis of dentinal tubule surface area using one-way ANOVA test |
|---------|-------------------------------------------------|
| | Dentinal tubule surface area |
| | Subgroup A | Subgroup B |
| | Mean (% area) | SD | Mean (% area) | SD |
| Group I | 18.7<sup>a</sup> | 2.1 | 23.04<sup>b</sup> | 2.3 |
| Group II | 42.9<sup>b</sup> | 5.6 | 29.03<sup>b</sup> | 2.7 |
| Group III | 33.4<sup>c</sup> | 4.4 | 31.1<sup>b</sup> | 3.1 |
| ANOVA P value | <0.001 | <0.001 |

All results are expressed as mean ± standard deviation (SD).
Not significant at P > 0.05. Significant at P < 0.05.
Means with same superscript letters in each column are not significantly different

| Table II | ANOVA statistical analysis of the EDAX results of Ca and P levels for enamel and dentin |
|---------|---------------------------------------------------------------------------------|
| | Enamel | Dentin |
| | Ca | P | Ca | P | Ca | P |
| Group I (Control) | | | | | | |
| Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| 20.54<sup>a</sup> | 0.25 | 18.41<sup>a</sup> | 0.30 | 19.92<sup>a</sup> | 0.11 | 17.95<sup>a</sup> | 0.25 |
| Group II (Acyanotic) | | | | | | |
| 18.59<sup>b</sup> | 0.26 | 16.12<sup>b</sup> | 0.16 | 17.92<sup>b</sup> | 0.01 | 15.29<sup>b</sup> | 0.20 |
| Group III (Cyanotic) | | | | | | |
| 18.73<sup>b</sup> | 0.41 | 16.58<sup>b</sup> | 0.33 | 17.63<sup>b</sup> | 0.43 | 16.01<sup>b</sup> | 0.12 |
| ANOVA P value | <0.001 | <0.001 | <0.001 | <0.001 |

All results are expressed as mean ± standard deviation (SD).
Not significant at P > 0.05. Significant at P < 0.05.
Means with same superscript letters in each column are not significantly different
enamel and dentin of groups II and III when compared with the control group. Statistical analysis using the one-way ANOVA test revealed a highly significant difference on comparing Ca and P levels of group II or group III with group I but there was no significant difference between group II and group III (Table II, Figs 3 and 4).

**Discussion**

Enamel has a protective role on the tooth; loss of enamel exposes the sensitive dentin underneath. Once damaged, enamel is generally unable to recover. Tooth enamel and/or dentin are also affected by systemic dis-
cases, the nature and severity of the disease itself as well as other factors often determine the extent of the damage to enamel and dentin. It is important for the dental practitioner to understand the nature of the underlying disease and the potential adverse effects that any therapy may incur [10].

Our study proves the presence of alterations in enamel and dentin of deciduous incisors obtained from CHD patients. These changes were observed in the ultrastructure as well as the mineral content (Ca and P) of enamel and dentin.

In the current work, numerous differences were found between acyanotic CHD specimens and healthy controls. ESEM analysis of enamel showed an increase in dissolution after preparation by etching. Also the number of dissolved prism heads increased, others manifested non-uniform outlines. Some specimens showed type III etching pattern. Prisms showed irregular orientation. These results were in agreement with Al-Etbi and Al-Alousi [11] whose results revealed that children with ventricular septal defect (acyanotic CHD) had a high percentage of enamel defects. Our findings may be explained by hypomineralization [12] of enamel of acyanotic CHD specimens. However, these results were found to disagree with Tasioula et al. [13] who concluded that no significant differences were demonstrated between CHD children and healthy children regarding enamel defects.

In the present study ESEM analysis for dentin of acyanotic specimens showed increase in lateral branching of dentinal tubules. Widening of dentinal tubules was evident. In addition, thinning of peritubular dentin was observed.

EDAX analysis of enamel and dentin of acyanotic CHD group confirmed the changes determined by ESEM analysis. Ca and P of enamel and dentin decreased significantly (P value < 0.001) indicating a degree of hypomineralization. These results were in agreement with those of a recent study conducted by Chico-Barba et al. [14], in which reduced bone quality was detected in CHD children.

In the present study the cyanotic CHD group showed several differences. In ESEM analysis the area of dissolution of enamel increased. Interprismatic substance showed increased dissolution and enamel prisms exhibited irregular orientation. These results agreed with Lygidakis et al. [15] who found that children with Molar-incisor-hypomineralisation present with medical problems during their prenatal, perinatal and postnatal period.

In the current investigation, the ESEM analysis showed dentinal tubule orifices with highly irregular outlines. The diameters of dentinal tubules presented a wide variation across the examined field, in addition to the presence of increased lateral branching. The intertubular dentin showed a highly granular appearance. These results were in agreement with Matsumoto et al. [16] who found that the cortical bone canals were larger in diameter, were more densely distributed and connected, and opened into the marrow cavity with a higher density in the chronic hypoxia group. They concluded that chronic hypoxia enhanced the formation of cortical canal networks at the postnatal developmental stage, probably facilitating intra- and transcortical vascularization and bone perfusion accordingly.

Affirmation of the reduction in mineralized structure of enamel and dentin was demonstrated by EDAX analysis. The difference in mineral content (Ca, P) of enamel and dentin between cyanotic and healthy groups was highly significant (P < 0.001), but there was not a significant difference between cyanotic and acyanotic groups. These results were in agreement with the findings of Rego et al. [17] who had assessed bone density in adolescents after surgical repair of tetralogy of Fallot (cyanotic CHD). The density of bone was found to be affected.

Conclusion

The results of the present study indicated that CHD leads to alteration in the structure of enamel and dentin of deciduous incisors on ultrastructural levels, in addition to a significant decrease in mineral content (Ca and P) (P < 0.001) of deciduous enamel and dentin when compared with healthy controls, rendering the dentition at increased risk of dental caries. Several disease-related factors may be involved in producing these changes; these include hemodynamic alterations, malnutrition, infective endocarditis, medication and hypoxia.

We recommend that cooperation between pediatric cardiologists and pediatric dentists should be increased. Spreading awareness of dental health and caries risk among CHD children and their parents must become a priority. More efforts for caries prevention must be directed towards CHD children, especially those in developing countries as they are at higher risk.

To the best of our knowledge the current work represents the first ultrastructural (micro-morphological) and chemical analysis of enamel and dentin of deciduous teeth of CHD children. This provided a unique opportunity to investigate the possible effects of CHD on deciduous teeth enamel and dentin, paving the way for further studies regarding the consequent implications in clinical practice.

**Funding sources:** None.

**Authors’ contribution:** BES – study concept and design, analysis and interpretation of data, statistical analysis, manuscript writing; GAA – study concept and design, full clinical and echocardiographic examination of patients, revised parts of the manuscript concerned with cardiol-
ogy; FMI and YMEH – study concept and design, study supervision, manuscript writing and revision. All authors had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest: None.

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