

Acid-etching effects in hypomineralized amelogenesis imperfecta. A microscopic and microanalytical study

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Received: 12-03-2004

Accepted: 18-05-2005

Sánchez-Quevedo C, Ceballos G, Rodríguez IA, García JM, Alaminos M. Acid-etching effects in hypomineralized amelogenesis imperfecta. A microscopic and microanalytical study. *Med Oral Patol Oral Cir Bucal* 2006;11:E40-3.

© Medicina Oral S. L. C.I.F. B 96689336 - ISSN 1698-4447

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Indexed in:

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ABSTRACT

Objectives: The purpose of this study was to use quantitative x-ray microprobe analysis with scanning electron microscopy to define the morphostructural and calcification patterns in the enamel of teeth with the hypomineralized variant of amelogenesis imperfecta.

Study design: We compared 5 fragments of permanent human canines from patients with clinically diagnosed hypomineralized amelogenesis imperfecta and 5 normal permanent canines from subjects without amelogenesis imperfecta. All specimens were etched with phosphoric acid for morphological and microanalytical examination.

Results: Two types of etching patterns were found; in addition, islets of pattern I were seen within areas of pattern II. Microanalysis detected no significant differences in calcium concentration between specimens with amelogenesis imperfecta and normal control specimens after acid etching. Pattern III was not observed.

Conclusions: The changes and their distribution in the enamel structure after 30 s of acid etching are described in teeth with this rare disorder. Although these data seem to coincide with alterations in prism development, no alterations in calcium concentration were found.

Key words: Acid-etching, microanalysis, quantitative method, scanning electron microscopy.

RESUMEN

Objetivos: El objetivo del presente trabajo consistió en definir los patrones morfológicos y de calcificación en el esmalte de dientes afectados con amelogénesis imperfecta hipomineralizada, tras la aplicación del grabado ácido, mediante la utilización de la microscopía electrónica analítica cuantitativa.

Diseño del estudio: Se estudian 5 fragmentos de caninos humanos permanentes de pacientes con amelogénesis imperfecta

hipomineralizada y 5 caninos controles. Todas las muestras fueron tratadas con ácido fosfórico y posteriormente procesadas para su estudio morfológico y microanalítico.

Resultados: Se observan dos tipos de patrones de grabados, I y II, así como áreas o islotes de patrón I insertados en extensas áreas homogéneas en las que predomina el patrón II. No se aprecian diferencias significativas en la concentración de calcio entre las muestras con amelogenesis imperfecta hipomineralizada y control después del grabado ácido tras el estudio microanalítico cuantitativo. El patrón tipo III no fue observado.

Conclusiones: Se describen los cambios y distribución en la estructura del esmalte después del grabado ácido durante 30 segundos en dientes con amelogenesis imperfecta hipomineralizada. Aunque los datos obtenidos coinciden con alteraciones en el desarrollo de los prismas, no se detectan alteraciones en la concentración de calcio.

Palabras clave: Amelogenesis imperfecta, patrones prismáticos, microscopía electrónica de barrido, microscopía electrónica analítica cuantitativa.

INTRODUCTION

Acid etching is important in odontology because it can serve as a model for the acid destruction caused by caries (1) and help in the taxonomic and evolutionary classification of mammals on the basis of structural differences in enamel (2). In addition, this technique is important in odontologic treatment because it facilitates the bonding of restorative materials to excavations and fissures it creates (3). Amelogenesis imperfecta is a term that identifies a group of hereditary alterations that affect the formation of the enamel extracellular matrix (4) by altering prism formation (5). Although odontological treatment for amelogenesis imperfecta varies depending on the patient's clinical status and the extent of the process itself, there are indications that acid etching can be used as a preliminary step in restorative treatment (6, 7).

The recent application of new electron microscopy quantitative analysis techniques to study mineralized tissues, allows us to establish a very closed relationship between the morphological and chemical patterns in those tissues (8-10). The application of analytical electron microscopy to odontology is an important advance to define the normal and pathological patterns of the mineralized dental tissues and the response of these structures to dental therapy (11, 12).

This study was designed to use quantitative x-ray microprobe analysis with scanning electron microscopy to characterize the structural and calcification patterns in the hypomineralized variant of amelogenesis imperfecta. The findings make it possible to analyze the effects of this technique on enamel prisms, and suggest how these effects might influence restorative treatments that use acid etching as a preliminary conditioning step.

MATERIAL AND METHOD

The material consisted of fragments of 5 permanent human canines from the coronal part of permanent teeth obtained in the course of preparation for crown replacement from patients with autosomal dominant clinically and genetically diagnosed hypomineralized amelogenesis imperfecta. All patients were members of the same family (13). As the control material we used 5 coronal fragments from normal included canines surgically extracted.

The tooth fragments were plunge-frozen in liquid nitrogen. The samples were transferred to Polaron E 5350 the freeze-drying apparatus and dried at -80°C for 24 h. The amelogenesis im-

perfecta fragments were subjected to acid etching with 35% phosphoric acid for 30 seconds, then washed in double-distilled water and air-dried for 1 h. Each fragment was mounted on an SEM stub and coated with carbon for microanalysis, and later with gold for morphological study with a Philips XL30 SEM and an EDAX DX-4 detector (14, 15)

Specimens prepared as described above were studied in a Philips XL-30 scanning electron microscope (SEM) (operating voltage = 15 kV; spot size = 500 nm; tilt angle = 35° ; take-off angle = 61.34°). An energy dispersive spectrometer (EDAX DX-4) was used for quantitative analyses (count rate = 1200 cps; live time = 50 s). Spectra were collected by pin-point electron beam at x 40 000, and ten analyses were run with each specimen, so that all 50 separate analyses were done in both amelogenesis imperfecta and control teeth.

The peak-to-background (P/B) ratio method (16, 17) was used to measure the concentration of calcium. Microcrystalline salt standards were used to quantify calcium (10, 15, 18, 19). Microcrystalline salts standards were methodologically treated in similar way to control and amelogenesis imperfecta specimens (20, 21). For statistical analyses we use the Student's t test.

RESULTS

Two types of etched patterns were seen in fragments of teeth with amelogenesis imperfecta. The type I pattern was characterized by deep central excavations and prominent margins (Fig. 1). The type II pattern showed a variable central protrusion and a notable degree of peripheral dissolution. The type II pattern was more widespread in the enamel structure than type I (Fig. 2). In areas where type I prisms were predominant, the size and appearance of the prisms were more variable than in type II. Acid etching also revealed prisms of variable size and appearance in the Retzius stria, but not in the areas between the Retzius lines, which were much more homogeneous in appearance. However, the effect of acid etching appeared to be similar in all prisms regardless of whether they were located within the Retzius stria or in the surrounding areas.

One of the most characteristic findings in specimens from amelogenesis imperfecta teeth was that acid etching revealed small areas ("islets") of type I alterations within large areas characterized mainly by the type II pattern (Fig. 3). The type I islets appeared to arise from mounds of amorphous material.

Percent weight fractions and mean weight fractions of calcium in the acid-etched enamel were 22.90 ± 2.52 in the type I pattern, 22.64 ± 1.95 in the type I pattern islets, and 21.95 ± 2.25 in the type II pattern of amelogenesis imperfecta samples. The values after acid etching in control specimens were 22.50 ± 2.41 in the type I pattern, and 21.61 ± 2.25 in the type II pattern. There were no statistically significant differences between amelogenesis imperfecta and normal teeth in calcium concentrations after acid etching.

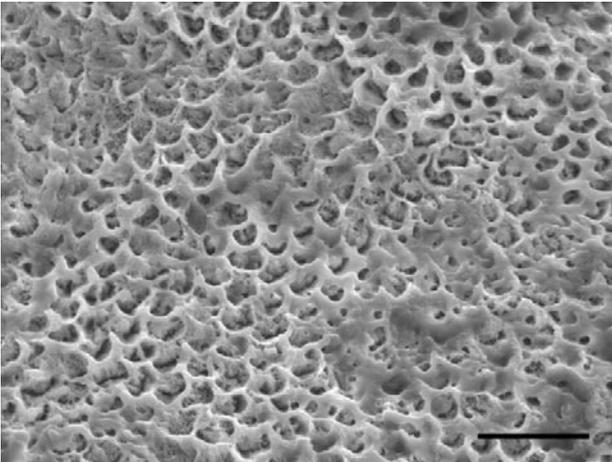


Fig. 1. The type I pattern in acid etched teeth with hypomineralized amelogenesis imperfecta was characterized by a deep central excavation and prominent margins. Bar, 20 μm .

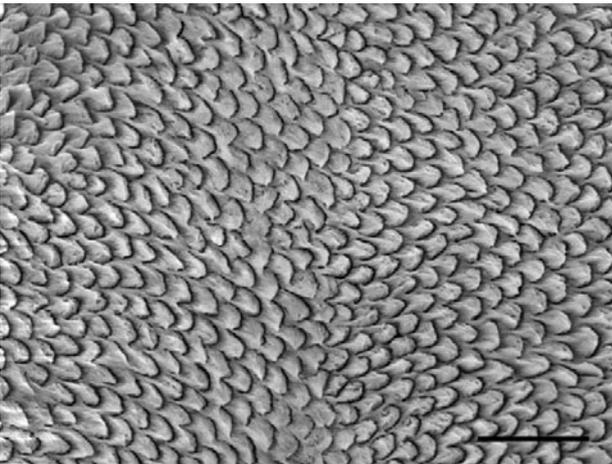


Fig. 2. The type II pattern, showing a variable central protrusion and a notable degree of peripheral dissolution. The Retzius stria is clearly visible in the center of the image. Bar, 20 μm .

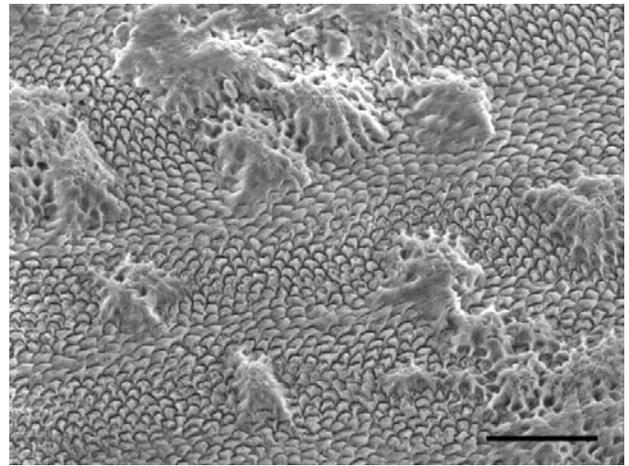


Fig. 3. Small areas ("islets") of type I alterations within larger areas characterized mainly by the type II pattern. Bar, 50 μm .

DISCUSSION

Acid etching and examination with scanning electron microscopy have revealed, in several species including humans, three main types of etching patterns (designated I, II and III) as described by Silverstone et al (22). The topographical distribution of the different patterns in the etched enamel has also been reported (23). In teeth fragments with clinically diagnosed hypomineralized amelogenesis imperfecta, we found type I and type II patterns, but not the type III pattern. This contrasts with the report by Seow and Amaratunge (7) who found all three patterns in teeth with amelogenesis imperfecta. In this connection it is important to note that these authors treated their specimens with acid for 1 min, a period that surpasses the maximum therapeutic exposure of 30 s.

Our finding of islets displaying the type I pattern within areas where the type II pattern predominated (as previously reported) suggests that alterations had occurred in prism development. This notion contrasts with the conclusions of Seow and Amaratunge (7) and Wright et al (11) who suggested that the presence of three different patterns in amelogenesis imperfecta was evidence of normal prism structure. The presence of islets meant that an alteration in the normal distribution of patterns established in controls and in previous studies (7-10) was present. In such studies our group has previously demonstrated that enamel prisms use to show parallel and decussated patterns, these latter being less frequent than the formers, and that independently from the spacial distribution, some prisms show filamentous patterns. Microanalytical study with quantitative electron microscopy of the calcium concentrations showed that there were no significant differences in the effects of acid etching between amelogenesis imperfecta and healthy control teeth. Our observations of the morphology and structure of the prisms suggest that acid etching has a more marked effect on the morphostructural features of the tooth enamel in hypomineralized amelogenesis imperfecta than on the mineral concentration as measured by microanalysis. Our studies with quantitative electron microscopy in hypomineralized amelogenesis imperfecta do not confirm the results obtained by Wright et al (24)

using qualitative electron microscopy. Our microanalytic results should be considered in the context of the reviewing that is currently being held about the mineralization process related to enamel and dentine in the different clinical modalities of amelogenesis imperfecta. These results should contribute to the current reviewing process of the amelogenesis classical clinical classification (19,25). Dentists should be aware that these effects may influence the outcome of restorative treatments that involve acid etching as a preliminary conditioning step. The knowledge of the acid-etching patterns of the different clinical types of amelogenesis imperfecta could help to prevent the high incidence of failure in the restorative treatments applied to these types of teeth (7).

REFERENCES

1. Boyde A, Jones SJ, Reynolds PS. Quantitative and qualitative studies of enamel etching with acid and EDTA. *Scanning Electron Microsc* 1978;II:991-1002.
2. Martin LB, Boyde A, Grine FE. Enamel structure in primates: A review of scanning electron microscope studies. *Scann Microsc* 1988;2:1503-26.
3. Retief DH. Effect of conditioning the enamel surface with phosphoric acid. *J Dent Res* 1973;52:333-41.
4. Dong J, Gu TT, Simmons D, Mac Dougall M. Enamel maps to human chromosome 4q21 within the autosomal dominant amelogenesis imperfecta locus. *Eur J Oral Sci* 2000;108:353-8.
5. Wright JT, Butler WT. Alteration of enamel proteins in hypomaturation amelogenesis imperfecta. *J Dent Res* 1989;68:1328-30.
6. Seow WK. Clinical diagnosis and management strategies of amelogenesis imperfecta variants. *Pediatr Dent* 1993;15:384-93.
7. Seow WK, Amaratunge FA. The effects of acid-etching on enamel from different clinical variants of amelogenesis imperfecta: an SEM study. *Pediatr Dent* 1998;20:37-42.
8. Sánchez-Quevedo MC, Ceballos G, García JM, Gómez de Ferraris MEG, Rodríguez IA, Campos A. Quantitative analytical electron microscopy of calcium in hypomineralized amelogenesis imperfecta. *Scanning* 23:2001;152-3.
9. Sánchez-Quevedo MC, Ceballos G, Rodríguez IA, García JM, Campos A. Quantitative X-ray microanalytical and histochemical patterns of calcium and phosphorus in enamel in human amelogenesis imperfecta. *International Journal of Developmental Biology* 2001;45:115-6.
10. Sánchez-Quevedo MC, Ceballos G, García JM, Rodríguez IA, Gómez de Ferraris MEG, Campos A. Scanning electron microscopy and calcification in amelogenesis imperfecta in anterior and posterior human teeth. *Histol Histopathol* 2001;16:827-32.
11. Wright JT, Duggal MS, Robinson C, Kirkham J, Shore A. The mineral composition and enamel ultrastructure of hypocalcified amelogenesis imperfecta. *J Craniofac Genet Dev Biol* 1993;13:117-26.
12. Zambrano M, Nikitakis NG, Sánchez-Quevedo MC, Rivera H. Oral and dental manifestations of vitamin D-dependent rickets type I: Report of a pediatric case. *Oral Surg Oral Med Oral Pathol Oral Radiol and Endodontics* 2003;95:705-9.
13. Ceballos A, Ceballos G. Estudio de una familia con amelogenesis imperfecta. *Avances en Odontostomatología* 1988;4 (3):125-8.
14. Campos A, Rodríguez IA, Sánchez-Quevedo MC, García JM, Nieto-Albano OH, Gómez de Ferraris ME. Mineralization of human premolar occlusal fissures A quantitative histochemical microanalysis. *Histol Histopathol* 2000;15:499-502.
15. Sánchez-Quevedo MC, Nieto-Albano OH, García JM, Gómez de Ferraris ME, Campos A. Electron probe microanalysis of permanent human enamel and dentine. A methodological and quantitative study. *Histol Histopathol* 1998;13: 109-13.
16. Statham PJ, Pawley JB. A new method for particle X-ray microanalysis on peak to background measurements. *Scanning Electron Microsc* 1978;1:469-78.
17. Small JA, Heinrich KFJ, Newbury DE, Myklebust RL. Progress in the development of the peak-to-background method for the quantitative analysis of single particles with the electron probe. *Scanning Electron Microsc* 1979;II:807-16.
18. López-Escámez JA, Campos A. Standards for X-ray microanalysis of calcified structures. *Scanning Electron Microsc* 1994; 8:171-85.
19. Sanchez-Quevedo MC, Ceballos G, Garcia JM, Luna JD, Rodriguez IA, Campos A. Dentine structure and mineralization in hypocalcified amelogenesis imperfecta: a quantitative X-ray histochemical study. *Oral Diseases* 2004;10: 94-8.
20. Warley A. Quantitative X-ray microanalysis of thin sections in biology: appraisal and interpretation of results. En: Sigeo DC, Morgan AJ, Sumner AT, Warley A, eds. *X-ray microanalysis in biology. Experimental techniques and applications*. Cambridge: Cambridge University Press; 1993. p. 47-57.
21. Warley A. *X-ray microanalysis for biologists*. London: Portland Press. 1997
22. Silverstone LM, Saxton CA, Dogon IL, Fejerskov O. Variation in the pattern of acid etching on human dental enamel examined by scanning electron microscopy. *Caries Res* 1975;9:373-87.
23. Risnes S. Multiplane sectioning and scanning electron microscopy as a method for studying the three-dimensional structure of mature dental enamel. *Scann Microsc* 1987;1:1893-902.
24. Wright JT, Deaton TG, Hall KI, Yamauchi M. The mineral and protein content of enamel in amelogenesis imperfecta. *Conn Tiss Res* 1995;32:247-52.
25. Aldred M, Savarirayan R, Crawford P. Amelogenesis imperfecta: a classification and catalogue for the 21st century. *Oral Diseases* 2003;9:19-23.

Acknowledgements

This study was supported in part by the Spanish Ministry of Education and Culture through Project no. PB97-0804, and by the Agencia Española de Cooperación Internacional through Project AEIC/98/01. We thank M. Ángeles Robles for her competent technical assistance.