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Scanning Electron Microscope [SEM] Study of Amelogenesis Imperfecta.

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Abstract

Objectives: The amelogenesis imperfecta (AI) is a heterogeneous group of heritable disorder. It is characterized by qualitative or quantitative anomalies of enamel. Two forms of AI are reported as syndromic and non-syndromic, of which latter form is the most prevalent. Amelogenesis imperfecta can have different inheritance patterns depending on the gene that is altered. Most cases are caused by mutations in the ENAM gene and are inherited as an autosomal dominant pattern. Amelogenesis imperfecta is also inherited as an autosomal recessive pattern and this form of disorder can result from mutations in the ENAM or MMP20 gene. The histopathologic features of AI are highly diverse and vary with each clinical type of AI. Aim of this study was to evaluate the ultrastructure of enamel rod pattern, interred zones and porus parts of the enamel in the deciduous and permanent teeth affected by Amelogenesis Imperfecta.

Material and methods; One deciduous and one permanent teeth were collected from the patient diagnosed with hereditary amelogenesis imperfect. Microstructural analysis was undertaken using a Jeol35 SEM fitted with the Deben Genie upgrade.

Results; SEM showed irregular enamel prism orientation and widened crystalline space in the enamel of affected teeth. Coating of the amorphous material on the surface of enamel rods was also seen.

Conclusion: Scanning electron microscopic investigation is one of the most effective methods in diagnosing and identifying the type of amelogenesis imperfecta.

Keywords; Amelogenesis imperfecta, Developmental disorder, enamel anamolies, SEM, Histopathology of AI,

Introduction

Highly mineralized structure in physical body is enamel, consisting of 96% of inorganic and 4% of organic components. 85% of its volume is occupied by hydroxyapatite crystals. The physical properties and physiological function of enamel are directly associated with the composition, orientation, disposition and morphology of the mineral component within the tissue [1] During the formation, enamel transitions from a soft and pliable form to its final form [2]. Ultimate composition of enamel may be a reflection of unique molecular and cellular activities that happen during its genesis. Deviation from this pattern may cause amelogenesis imperfecta. [3]. Amelogenesis imperfecta (AI) may be a heterogeneous group of heritable disorder. It is characterized by qualitative or quantitative anomalies of enamel. Two sorts of AI are reported as syndromic and non-syndromic, of which latter form is that the most prevalent. Amelogenesis imperfecta can have different inheritance patterns counting on the gene that's altered. Most cases are caused by mutations within the ENAM gene and are inherited as an autosomal dominant pattern. Amelogenesis imperfecta is additionally inherited as an autosomal recessive pattern and this type of disorder may result from mutations within the ENAM or MMP20 gene [4] .The AMELX, ENAM, KLK-4 and MMP20 genes provide instructions for creating proteins that are essential for normal tooth development. These proteins are involved within the formation of enamel, which may be a hard, calcium-rich material that forms the protective outer layer of every tooth. Mutations in any of those genes alter the structure of those proteins. As a result, enamel is abnormally thin or soft and would have yellow or brown color [5]. Teeth with defective enamel are weak and simply damaged. The histopathologic features of AI are highly diverse and vary with each clinical sort of AI [6]. Scanning microscopy investigation is one among the foremost effective methods in diagnosing and identifying the sort of amelogenesis imperfecta. Aim of this study was to gauge the enamel rod pattern within the deciduous and permanent teeth suffering from Amelogenesis Imperfecta. This paper describes [SEM] ultrastructural study done on extracted teeth of non-syndromic amelogenesis imperfecta case.

Material and methods

A 16 years old female patient reported with a chief complaint of discolored and malaligned teeth with halitosis. She had psychosocial impact caused due to her dental esthetics. The parents of the patient revealed that those yellow stains on her teeth have been present since early childhood. Further they revealed similar discolorations of teeth in her family with her brother, and father [Fig.1] Indicating a genetic basis of discoloration. She had consonant smile and 100%incisor exposure with gummy smile, and pigmented gingiva. The intra-oral examination revealed generalized yellowish discoloration of teeth along with diffuse pitting, seen more prominently on the labial and buccal aspect of the teeth**.** The surfaces of the teeth were rough and irregular in shape [Fig.2 A]**.** Average sized arches with interdental space between the teeth were observed. Palatally erupted 22, over retained upper and lower right canine, anterior bidental crowding seen. [Fig.2 C, D] She had Class II molar on left side and end on right side on skeletal bases, Anterior cross bite, edge to edge bite and skeletal class III relation [Fig.2 B]. On palpation of teeth with probe, tooth material was soft in consistency with mild flaking of residual enamel**.**

Radiograph showed impacted 13, 43, generalized thinning of enamel surface along with absence of enamel at some regions [Fig 3.] indicating disturbed morphology of teeth. Considering all the clinical and radiological findings case was diagnosed as amelogenesis imperfecta. As the part of orthodontic and prosthodontic treatment plan the deciduous teeth and impacted canines were extracted and subjected for SEM study.

Direct and Indirect are the two methods for carrying out SEM study. Present study was carried out with direct method. As the direct method requires an effective dehydration and drying procedure to avoid artefact, a simple, fast and very effective procedure of direct method, is followed in this investigation. Dehydration is carried out with differently concentrated mixtures of water/ethanol followed by mixtures of ethanol/acetone so that critical point drying can be avoided with regard to hard tooth tissues. Extracted deciduous and permanent teeth were fixed in 2.5% glutaraldehyde for 24 hours. Teeth were coated with Ag by Ion beam sputtering [Fig.4]. Specimen were kept in vacuum for 8 hours and then mounted on the cassette and placed in SEM [Fig 5]. Microstructural analysis was undertaken using a Jeol 35 SEM fitted with the Deben Genie upgrade[Deben Engineering, Debenham, UK] [Fig.6].

Results

SEM examination of sections of affected teeth revealed loss of normal enamel architecture [Fig. 7C], which in part reflected the presence of an amorphous material obscuring the enamel rods. It was typically distributed over most of the enamel. Scanning Electron Microscopy showed incompletely formed enamel rods [Fig. 7A]. Irregular enamel prism orientation and widened crystalline space in the enamel of affected teeth [Fig. 7C]. Underlying dentin appeared normal [Fig. 7D]..Considering all the above mentioned findings the present case was classified as autosomal dominant (AD)-inherited hypocalcified AI.

Discussion

Amelogenesis imperfecta is a developmental disorder, often inherited and affects the dental enamel. It usually occurs in the absence of systemic features and comprises of diverse phenotypic entities [2]. AI is caused by mutations or altered expression in five genes: AMEL (amelogenin), ENAM (enamelin), MMP20 (matrix metalloproteinaise-20), KLK4 (kallikrein-4) and FAM83H. [7]. Ameloblasts control the secretion, formation and maturation of the enamel matrix using matrix molecules such as enamelin, amelogenin, ameloblastin, tuftelin, amelotin, dentine sialophophoprotein and matrix enzymes such as kallikrein-4 and matrix metalloproteinaise-20. [7]. Diversity of enamel malformations observed in AI is believed to reflect the differences in the timing during amelogenesis, when the disruptions occur. Defects during the formation of DEJ can tear the enamel layer easily from dentin, which will lead to hypoplastic enamel and any flaws during the maturation stage, such as, if the enamel matrix is not properly degraded and reabsorbed, produce an enamel layer that is of normal thickness, but is pathologically soft[7]. Similar kind of enamel was seen in our patient.

The predominant clinical manifestations of affected individuals are enamel hypoplasia (enamel is seemingly correctly mineralized, but thin), hypomineralization (subdivided into hypomaturation and hypocalcification), or a combined phenotype [8]. Hypoplastic AI represents 60 to 73% of all cases, hypomaturation AI represents 20 to 40%, and hypocalcification AI represents 7%. In comparison with hypomaturation type, the mineralization in this type is markedly reduced. Clinically, the crowns of the teeth in such cases appears to be opaque white to

yellow-brown, soft rough enamel surface, dental sensitivity and very poor aesthetics. Due to severe hypomineralization, there may be early loss of enamel. The thickness of enamel appears to be normal at eruption that often chips and tends to abrade easily post eruptively. There may be delayed eruption of teeth. Accumulation of a large amount of supragingival calculus is evident, and such similar findings were seen in our case.

The phenotype (clinical appearance) of AI shows a spectrum of clinical variability and this depends on the pattern of inheritance, the mutation involved, expression of matrix proteins and biochemical changes associated with the mutations [7]. The characteristics of hypomaturation AI are (i) Enamel of normal thickness but mottled in appearance (ii) Slightly softer than normal and vulnerable to tooth wear, but not as severe as the hypocalcified type (iii) Radiographically, similar radio density as dentin.[7,9]. Likewise the clinical presentation of our case was similar to the features described by K.Gadhia et al.

The trait of AI can be transmitted by an autosomal-dominant, autosomal-recessive, or X-linked mode of inheritance.[6,] In cases, with an X-linked form, the disorder results from a mutation in the amelogenin gene, AMELX. In cases of dominant forms of AI, the enamelin gene, ENAM, is implicated in the pathogenesis [10]. The hypo calcified type shows pigmented and easily detachable enamel. Radiographically, enamel thickness is normal, but its density is even less than that of the dentin [11]

Various classification systems have been described in the literature but widely used is Witkop 1988 [12].

Type I	Hypoplastic
IA Hypoplastic pitted autosomal dominant IB Hypoplastic local autosomal dominant IC Hypoplastic Local autosomal Recessive ID Hypoplastic smooth autosomal dominant IE Hypoplastic smooth X- linked dominant IF Hypoplastic rough autosomal dominant IG Enamel agenesis, autosomal recessive	
Type II	Hypomaturation
IIA Hypomaturation pigmented autosomal recessive IIB Hypomaturation X-linked recessive IIC snowcapped teeth, autosomal dominant	
Type III	Hypocalcified
IIIA Autosomal dominant IIIB autosomal recessive	
Type IV	Hypomaturation- hypoplastic with taurodontism
Type IV A Hypomaturation- hypoplastic with taurodontism, autosomal dominant Type IVB Hypoplastic- hypoplastic with taurodontism, autosomal dominant	

Scanning electron microscopic study of the affected tooth helps us to understand the microstructure in detail and also to identify the type of AI. SEM findings of our case showed incompletely formed enamel rods, widened crystalline space in the enamel of the affected teeth and loss of enamel architecture was observed. The dentin was normal in SEM appearance. The similar type of findings on SEM were found in the study conducted by Sayed et al, they found poorly formed enamel rods with inappropriate retention of amorphous material, which is likely to represent retained organic matrix that contributes to the overall hypomineralised phenotype [13].Wright et al. evaluated the primary teeth affected from hypoplastic AI

by using SEM and visualized irregular outlines in surface prism structure, and did not encounter any pitting of surface enamel defects [14]. In another study, the poor orientation of crystallites and empty zones between the crystallites, were observed in hypoplastic type AI cases [15, 16]. Backman et al. examined the primary teeth with hypoplastic AI and observed irregular hypoplastic areas and depressions on the enamel surfaces [17].

Aldred et al. evaluated the exfoliated primary teeth of children with AI and informed that the teeth featured multiple shallow depressions and deep tubular voids on the enamel surfaces. They thought that the reason of the depressions and voids could be the defective protein synthesis and/or mineralization [18]. The similar finding were seen in our study.

R.C. Shore et al in their original paper did SEM and X-ray spectroscopy 3 deciduous teeth in one family and SEM of tooth sections revealed disrupted prism morphology and the prisms had a glass-like appearance in some areas. These areas of dysplasia were sometimes irregular but formed regular arrays in others [19]. In the present study, scanning Electron Microscopy showed irregular enamel prism orientation and widened crystalline space in the enamel of affected teeth. Coating of the amorphous material on the surface of enamel rods was also seen.

Seymen et al evaluated the enamel surfaces of exfoliated primary teeth of three children with AI using SEM in their study, and observed disoriented enamel prisms, deep tubular voids on the enamel surfaces. Amelogenesis imperfecta affects the enamel crystallites so they form abnormal crystallite morphology [20]. With the help of SEM findings we could diagnose the case as type II AI. The patient underwent oral prophylaxis and orthodontic treatment for malaligned teeth

Conclusion

Scanning electron microscopic investigation is one of the most effective methods in diagnosing and identifying the type of amelogenesis imperfecta. Progress in the ongoing molecular, biochemical and histological research will improve the ability to diagnose amelogenesis imperfecta and, as a consequence, the quality of the treatment. Scanning electron microscopic [SEM] investigation is one of the most effective methods in diagnosing and identifying the type of amelogenesis imperfecta.

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Legends Figure

Fig.1: affected father, son and daughter showing hereditary involvement of AI.

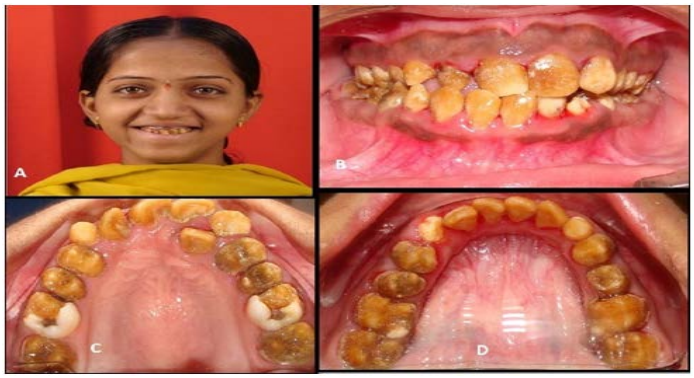

Fig.2 A; Frontal profile showing discoloration, B; malalignment of teeth, C &D; over retained canine and crowding.

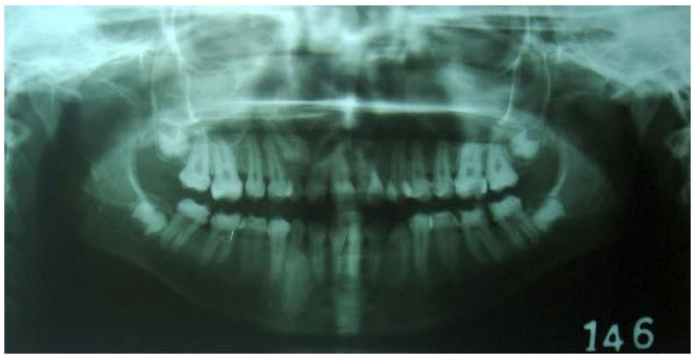

Fig.3: OPG showing impacted teeth and thin layer of enamel

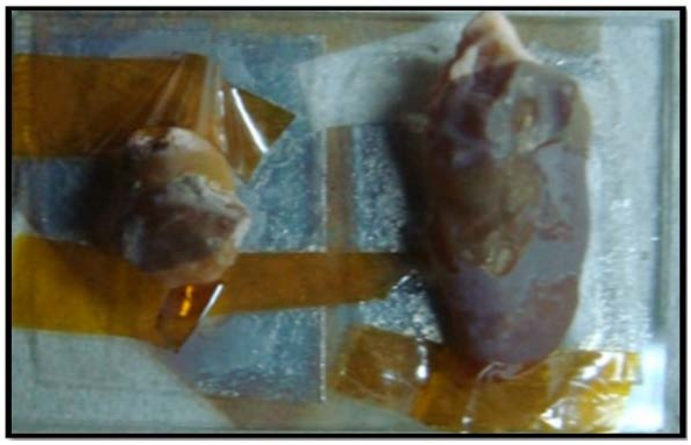

Fig.4: Teeth coated with Ag by Ion beam sputtering

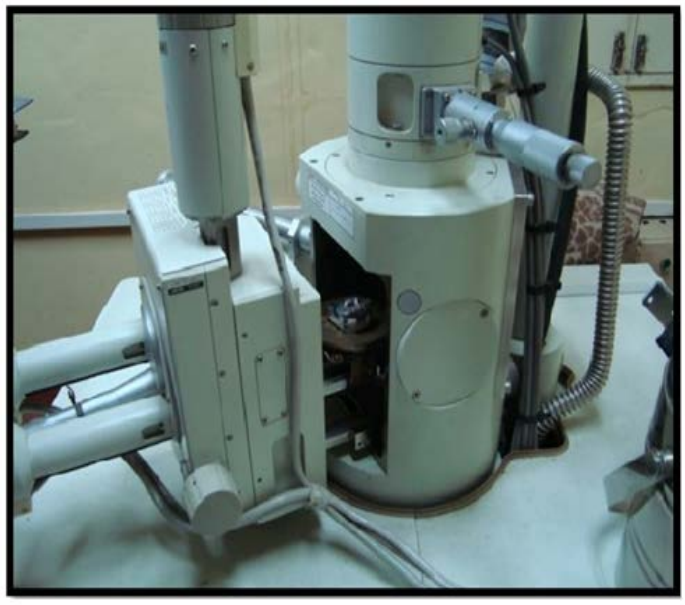

Fig.5: Teeth mounted on the cassettes and placed in SEM

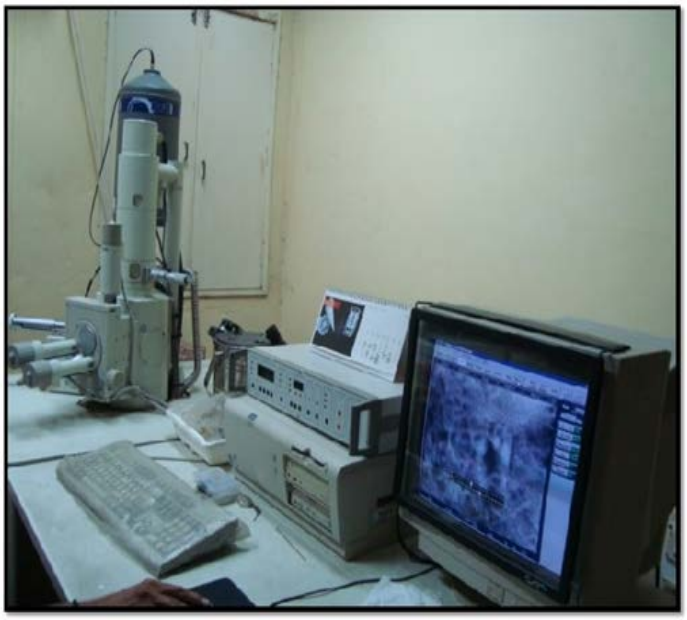

Fig.6: Microstructural analysis using Jeol 35 SEM fitted with the Deben Genie upgrade

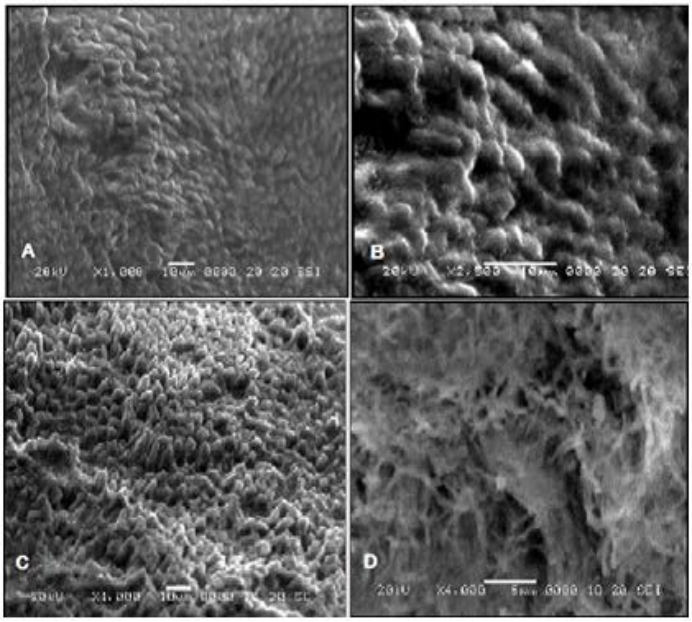

Fig.7: SEM showing A: incompletely formed enamel rods B: loss of normal enamel architecture C: widened crystalline space in the enamel of affected teeth D; Normal dentin.

Classification of AI according to Witkop (1988);	
Type I	Hypoplastic
IA Hypoplastic pitted autosomal dominant IB Hypoplastic local autosomal dominant IC Hypoplastic Local autosomal Recessive ID Hypoplastic smooth autosomal dominant IE Hypoplastic smooth X- linked dominant IF Hypoplastic rough autosomal dominant IG Enamel agenesis, autosomal recessive	
Type II	Hypomaturation
IIA Hypomaturation pigmented autosomal recessive IIB Hypomaturation X-linked recessive IIC snowcapped teeth, autosomal dominant	
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Type IV	Hypomaturation- hypoplastic with taurodontism
Type IV A Hypomaturation- hypoplastic with taurodontism, autosomal dominant Type IVB Hypoplastic- hypoplastic with taurodontism, autosomal dominant	

Table 1: Witkop classification of AI