MORPHO-HISTOLOGICAL AND BIOCHEMICAL STUDY OF ENAMEL IN SOUND AND HYPOMINERALIZED PERMANENT FIRST MOLARS

Rabab Mubarak* and Walid Ali**

ABSTRACT

Background: Enamel hypomineralization is the most common developmental disorder observed in teeth. It has general characteristic features and lower mechanical properties, but there has been little work on the morpho-histological features and chemical composition of hypomineralized molars. The aim of this study was to determine morphological characteristics, histological and ultrastructure features and chemical composition of sound and hypomineralized enamel of permanent first molars.

Methods: ten permanent first molars were used in this study. Seven of them were affected by enamel hypomineralization and used as experimental group and the remaining three were sound, and used as control group. The morphological characteristics were examined and photographed using Sterio microscopy. Longitudinal sections were prepared and histological features were detected. The ultrastructure of sound and hypomineralized enamel was examined using SEM. The chemical composition was also detected using EDX.

Results: Enamel hypomineralization appeared as white demarcated or diffuse opacities. It varied in size, shape and site and was characterized by absence of perikymata. Histologically, the hypomineralized enamel revealed normal and regular thickness. It followed the lines of Retzius but obscuring the characteristic dark and light bands of Hunter Schreger. SEM examination showed that, the hypomineralized enamel composed of irregular and disordered enamel prisms with indistinct boundaries and wide interprismatic zones. The enamel prisms appeared disorganized with loosely packed hydroxy apatite crystals. EDX analysis revealed that, the Ca/P ratio was higher in the sound enamel than the hypomineralized enamel. While the O/C ratio was higher in the hypomineralized enamel than in sound enamel.

KEYWORDS: Enamel hypomineralization; histological features; ultrastructure; chemical composition.

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INTRODUCTION

The developmental dental defects of enamel can be classified as quantitative enamel hypoplasia or qualitative enamel hypomineralization (1). Isolated developmental enamel defects are commonly seen in first permanent molar teeth, with up to 19.3% of children affected (2). Hypoplasia is caused by a disruption to the ameloblasts during matrix secretion and results in a quantitative defect of the enamel. While disruption that occurs during either the calcification or maturation phase of amelogenesis, results in a qualitative hypomineralized defect in the enamel (3). The classification of developmental enamel defects into Hypoplasia and hypomineralization is based on descriptive criteria and no association is made to etiology (4).

Hypomineralization of the enamel is the most common developmental disorder observed in teeth. The prevalence of this kind of hypomineralization is about 10-19%. These hypomineralized molars are often referred to as cheese molars, because the lesions clinically resemble cheese in color and consistency. Other descriptions are: idiopathic enamel hypomineralization, Idiopathic enamel opacities, Non-endemic mottling of enamel or Molar-Incisor Hypomineralization as incisors can also be affected but to a lesser degree than the molars (5). Today the proposed expression for the disease is Molar-Incisor Hypomineralization (MIH) (6). Clinically, hypomineralized enamel can be seen as either white-yellow or yellow-brown demarcated opacities or diffuse opacities, which are typical for fluorosis. Well-mineralized enamel is translucent, while hypomineralized enamel shows abnormal translucency (7). Hypomineralized enamel exhibits lower mechanical properties as lower hardness values and lower modulus of elasticity compared to normal enamel (8).

When several molars are affected with enamel hypocalcification, there is a great risk of incisors affection (MIH) (9-11). These findings indicate that the origin of (MIH) is a systemic factor during the first year of life and have a specific influence on the development of enamel during a limited period of time. With the exception of enamel alterations in hereditary conditions such as amelogenesis imperfecta (12&13) and epidemiolysis bullosa (14) or in conditions caused by known etiological agents such as fluoride (15-17), several etiological factors can cause these defects. Some studies have shown a relation between intakes of dioxins via mother’s milk after prolong breast-feeding. Complications involving oxygen shortages during birth or respiratory diseases such as asthma, bronchitis or pneumonia are considered as further etiological factors. Renal insufficiency, hypoparathyroldism, diarrhea, malabsorption and malnutrition and high fever diseases can be other reasons for the occurrence of these defects (18-22). A preliminary hypomineralization severity index was developed for dentitions with hypomineralized first permanent molars in children. This index has indicated great associations between hypomineralization of molars and some medical conditions, particularly implicating fevers, chicken pox, prenatal conditions and antibiotic use (23). Defective enamel can be a site of lower resistance for caries and it also tends to be hypersensitive that leads to increased levels of children anxiety during dental treatment (2, 24 & 25). The defective enamel can fracture easily under normal occlusal forces leading to excessive shipping, wear, and improper cavity preparation and sever restorative problems (2, 10, 26).

Most of studies on enamel hypocalcification have focused on its prevalence, etiology and mechanical properties but there has been little work on
the morphological and histological features of the hypomineralized teeth. Such findings may help in defining proper treatment strategies and improving the restorative outcomes of these teeth. So that, the aim of this study was to determine the morphological and histological characteristics of sound and hypomineralized enamel of permanent first molar by means of Sterio microscopy, determine the ultrastructure features of the sound and hypomineralized enamel using scanning electron microscopy (SEM) and finally to determine the chemical composition of sound and hypomineralized teeth using Energy Dispersive X-ray Analysis (EDX).

MATERIALS AND METHODS

Ten permanent first molars were used in this study. Three of them were sound, characterized by clinically normal mineralized enamel and used as control (group I). The remaining seven molars were affected by varying degrees of enamel hypomineralization with or without occlusal caries were used as experimental group (group II). These molars were early extracted from young boys (7-9 years old) who indicated for removal of their four first permanent molars (with incompletely formed roots) to allow their replacement by the 2nd permanent molars with the expectation that a more symmetrical dentition will result as described by pedodontics and orthodontics treatment planning for these cases. The extracted molars were stored in buffered solution (4.6 formaldehyde solution).

1-Morphological examination

The outer surfaces of the intact hypomineralized molars and sound molars were examined and photographed using Sterio microscopy (Leica S8Apo) to reveal their morphological characteristics. The buccal, lingual, mesial and distal surfaces of the hypomineralized molars were examined while the occlusal surfaces were excluded due to enamel disintegration or caries.

2- Histological and ultrastructure examination:

Each tooth was encased in cold cured epoxy resin and cut sagitally buccolingual in the axial plane using a water-cooled diamond disc into two halves (mesial and distal). Each half was kept without polishing. The sectioned surfaces were examined and photographed using the Sterio microscopy to reveal the histological features of sound and hypomineralized enamel. The mesial halves of sound and hypomineralized molars were etched with 30% phosphoric acid for 30 sec for disclosure of ultrastructural details at the prism and crystal levels. The specimens were carefully rinsed with distilled water then left to dry 24 h. The dehydrated specimens were sputter coated with gold and examined using SEM (Electronic Probe Microanalyser Jeol JAX-840A) to reveal the ultrastructure of the sound and hypomineralized enamel.

3- Chemical composition examination:

The distal halves of the sound and hypomineralized molars were used for measuring of the percent component composition using Energy Dispersive X-ray Analysis (EDX). X-ray detector system (EDX, INCA-X-sight) attached to a SEM (Jeol JAX-840A) was used. Three counts were conducted for the normal enamel (control) at different three areas in the center of the distal surface and other three counts were conducted for the hypomineralized enamel at different three areas at the margins and in the center of the hypomineralized lesion.
RESULTS

1- Morphological result

Light microscopic examination of the intact 1st permanent molar teeth using Sterio Microscopy revealed that, the normal enamel of the sound molars (control group) was uniform in color and translucent. The enamel of buccal and lingual surfaces was characterized by presence of group of transverse ridges (perikymata) that represented the enamel rods on the tooth surface. The enamel of the hypomineralized molars appeared as white demarcated or diffuse opacities. The demarcated opacities had either well or ill-defined margins with the surrounding normal enamel. The hypomineralized areas were characterized by absence of perikymata (Fig. 1 &2). The occlusal surfaces were always excluded from the study due to enamel disintegration particularly at cusps and ridges. The margins of the disintegrated areas were hypo mineralized and irregular (Fig. 3). Enamel hypomineralization varied in size, shape and site. It might affect all the surfaces of the tooth or one or more surfaces might be sound. The localized opacities appeared on the occlusal, middle or cervical thirds of each surface.

2-Histological and urtlesstucture results

Sterio microscopic examination of the longitudinally sectioned molars showed that, the normal enamel was uniform, regular and translucent. The lines of Retzius were well defined. The alternating dark and light bands of Hunter Schreger were detected at the cervical part of the longitudinally sectioned enamel. The hypomineralized enamel of localized or diffuse opacities had normal and regular thickness. The hypomineralized areas extend from the cusps cervically. It varied in size, shape and site. It might involve the whole enamel thickness from the outer surface to the dentino-enamel junction or it might involve only the external part of enamel. Localized opacities were detected at occlusal, middle or cervical parts of the enamel. The localized and diffuse opacities followed the lines of Retzius but obscuring the characteristic dark and light bands of Hunter Schreger (Fig. 4).

Scanning electron microscopic (SEM) examination of the etched enamel surface of permanent first molars revealed that, the normal enamel composed of uniformly, well arranged enamel prisms (rods) surrounded by prism sheaths. The prism structure was well organized and tightly packed with properly oriented hydroxyapatite crystals. There was minimal porosity (Fig 5&6).

Fig(1): A photomicrograph of 1st permanent molar showing sound translucent enamel and pronounced perikymata (a) and hypomineralized opaque enamel with loss of perikymata (b) (Sterio microscopy X 10).

Fig(2): A photomicrograph of hypomineralized 1st permanent molar showing localized white opacity with well-defined margin (a), localized white opacity with ill-defined margin (b) and diffuse white opacity(c) (Sterio microscopy X 10).
The hypomineralized enamel composed of irregular and disordered enamel rods with indistinct boundaries and wide interprismatic zones. The enamel prisms appeared disorganized with loosely packed hydroxyapatite crystals. There were obvious pores (Fig. 7&8).

3- Chemical composition (EDX) results

The results of EDX analysis for the chemical composition of sound and hypomineralized enamel are shown in figure (9) and statistical analysis are shown in table (1).

The table presents the means of weight percent (wt %) of Calcium (Ca), Phosphorous (P), Oxygen (O) and Carbon (C) in sound and hypomineralized enamel. The mean of the Ca/ P ratio and the mean of O/C ratios were calculated. Results showed that, the weight percent of Ca and P was higher in normal enamel compared with hypomineralized enamel. The Ca/ P ratio was lower in the hypomineralized enamel than in the normal enamel. While the O/C ratio was higher in the hypomineralized enamel than in the normal enamel.
In this study, it was interesting to find a permanent 1st molar affected by enamel hypomineralization and its contra lateral molar was sound in the same individual. This finding has emphasized the reports of other studies. A typical feature of hypomineralized molars was the asymmetrical appearance, that is the enamel of one molar could be severely affected while the enamel of contra lateral molar was clinically unaffected or having only minor sub surface defect \(^{(26)}\). This finding of affecting homologous teeth to varying extents might be due to short duration of the affecting insult, just affecting ameloblasts to a certain stage of development\(^{(27)}\).

**TABLE (1)** Shows the results of EDX analysis of sound and hypomineralized enamel.

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**DISCUSSION**

Fig (7): A SEM photomicrograph of hypomineralized enamel of 1st permanent molar showing irregular disordered enamel prisms (a) and wide interprismatic areas (b) (SEM X 3000).

Fig (8): A higher magnification of the previous SEM photomicrograph showing disorganized and loosely packed hydroxyapatite crystals with obvious porosity (SEM X 6000).

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Enamel hypomineralization has been considered to be a qualitative change of the matrix produced by disorders of the ameloblasts at the stage of maturation. Followed by recovery of some affected secretory as well as maturative cells (28).

Enamel formation was described as one stage of matrix formation and three phases in the stage of maturation (early, late and final). In the early maturation phase, the enamel is dull, white and relatively soft. While, in the late maturation phase the deeper enamel is well mineralized but the outermost layer is still incompletely mineralized and its mineral content increases during the final phase of maturation (29). Ameloblasts are very sensitive to disorders at an early stage of maturation. If the cells are damaged by systemic or local disorder at this stage it can’t easily recover from dysfunction during the long period of maturation. The sensitivity to systemic disorders and the mode of reaction of ameloblasts are not the same for each of the stages of the enamel development. Ameloblasts affection at the early stage of maturation resulted in prominent hypomineralization. On the other hand, disorders that occurred at the stage of matrix formation resulted in highly mineralized enamel (29 & 30).

In this study, morphological examination of hypominalized enamel revealed presence of white demarcated or diffuse opacities. The hypomineralized areas were characterized by absence of perikymata. These results are in agreement with previous studies that described the macroscopic appearance of enamel hypomineralization. They found white, creamy-yellow or yellow demarcated opacities and diffuse patchy opacities. Hardness values were related to color change, with yellow lesions being softer than white (31). In contrast to our description of demarcated opacities that characterized by loss of perikymata on the hypominalized surfaces, other studies have described the histomorphological appearance of hypomineralization in dental fluorosis. They reported that, fluorotic enamel appeared as porous areas along the striae of Retzius giving rise to accentuated perikymata (32). The demarcated opacities appeared to have well defined margins with the surrounding normal enamel. This finding referred to presence of group of impaired cells and neighboring cells having a normal function. This indicated that cells in a specific stage of development are affected (27).

Morphological examination of hypominalized areas in each tooth surface revealed variation in size and shape. This finding might illustrate different degrees of mineralization and variations in the sensitivity of ameloblastic response to different insults. This might also indicate temporary disturbance of ameloblasts that followed by its recovery.

Histological examination of the longitudinally sectioned molars showed that, the hypomineralized enamel of localized or diffuse opacities had normal and regular thickness. It extended from the cusps cervically. It varied in size, shape and site. A few studies have dealt with histomorphological appearance of demarcated opacities of enamel. The hypomineralized lesions of demarcated opacities were divided into 3 groups according to their size, position and shape. The 1st group (40%) involved the whole or nearly whole, the thickness of enamel and was usually located on the side of the enamel. The 2nd group (40%) was within the cuspal enamel, while the 3rd group (20%) appeared as a narrow band lying close to the outer surface (33). However, in this study the localized opacities were detected at occlusal, middle or cervical parts of the enamel. On the contrary, a previous study using polarized light microscopy has revealed that, only the
occlusal part was affected by hypomineralization and never cervical part was being affected. They referred that to potential disturbance of the insult to ameloblasts during the child’s early years. Another possible explanation was that, the thickness of enamel influenced the possibility for the ameloblasts to resist the insult \(^{(27)}\). The localized and diffuse opacities followed the lines of Retzius but obscuring the characteristic dark and light bands of Hunter Schreger. This might be due to the loss of the translucent nature of sound enamel so that, the opacity of hypomineralized enamel interfered with the bands of Hunter Schreger that was basically an optical phenomenon affected by the passage of light through the enamel.

Scanning electron microscopic examination showed the basic structure of the etched enamel surface of permanent first molars. It revealed that, the normal enamel composed of uniformly, well arranged enamel prisms (rods) surrounded by prism sheaths. The prism structure is well organized and tightly packed with properly oriented hydroxyapatite crystals. There was minimal porosity. While the hypomineralized enamel composed of irregular and disordered enamel prisms (rods) with indistinct boundaries and wide interprismatic zones. These findings are supported by other TEM study that showed wider sheath regions and larger gaps between prisms in hypomineralized enamel than normal enamel \(^{(34)}\). In this study, the enamel prisms appeared disorganized with loosely packed hydroxyapatite crystals. There were obvious pores. These results were in accordance with other SEM studies \(^{(1, 8, 27, & 31)}\). The increase in porosity and disorganization might contribute to the reduction in their mineral content \(^{(8)}\).

The featureless and amorphous appearance of hypomineralized porous enamel of the demarcated opacities might be due to poor acid etching. The reason for poor acid solubility could be explained by a higher content of organic matter (proteins) in the hypomineralized areas as proteins might reduce access of inorganic ions of acid etching to the crystallite surfaces \(^{(1 & 34)}\). In addition, the SEM results confirmed the morphological results. The severe loss and disintegration of the hypomineralized enamel mostly at the occlusal surfaces shortly after eruption is probably due to the loose arrangement of the apatite crystals particularly at the sheath region. Similarly, the optical opacities (diffuse or demarcated) associated with the hypomineralized enamel may also be attributed to the lower refractive index of the less dense structure of the enamel rods that increases the amount of light scattering within the enamel \(^{(35)}\).

Results of EDX analysis confirmed the SEM findings. It showed that, the weight percent of Ca and P was higher in sound enamel compared with hypomineralized enamel. The Ca/P ratio was higher in the sound enamel than the hypomineralized enamel. While the O/C ratio was higher in the hypomineralized enamel than in the sound. These results assured other previous findings. X-ray microanalysis of hypomineralized enamel revealed presence of higher content of carbon and lower concentration of Ca and P than normal enamel. The mean Ca/P ratio was lower in hypomineralized areas than in the adjacent normal enamel \(^{(36)}\). Similarly, a reduction in mineral density of hypomineralized enamel has been reported using microcomputer tomography imaging\(^{(37)}\). In addition, X-ray diffraction analysis showed no alteration in the calcium mineral phase in the hypomineralized enamel. The only calcium phosphate phase present in the hypomineralized enamel was calcium apatite. This suggested no substitution of any other ions.
such as Mg for Ca. It has been reported that the mechanical properties of a calcified tissues are generally linked to their mineral content. Previous studies have measured the hardness and modulus of elasticity of hypomineralized enamel. They found statistically lower mechanical properties in hypomineralized enamel than normal enamel which was attributed to a significant reduction in mineral content of hypomineralized enamel beside the looser prism structure.

In conclusion, hypomineralized enamel was characterized by many different morphological and histological features in comparison to normal enamel. The SEM examination and EDX analysis were also significantly different in hypomineralized enamel.

REFERENCES


