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Pre and Post Natal Enamel Mineralization in Primary Teeth from Children with Congenital Insensitivity to Pain with Anhidrosis (CIPA)

Arwa Gera[1], Lipa Bodner[2], Eliyahu Mass[1] and Uri Zilberman[3]*

[1]Pediatric Dental Clinic, Barzilai Medical University Center, Ashkelon, Affiliated to Ben-Gurion University of the Negev, Beer-Sheva, Israel

[2]Head of Oral and Maxillofacial Surgery, Division of Surgery, Ben-Gurion University of the Negev, Beer-Sheva, Israel

[3]Head of Pediatric Dental Clinic, Barzilai Medical University Center, Ashkelon, Affiliated to Ben-Gurion University of the Negev, Beer-Sheva, Israel

***Corresponding Author:** Uri Zilberman, Head of the Pediatric Dental Clinic, Barzilai Medical University Center, Ashkelon, Israel.

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Abstract

Enamel mineralization is affected by genetic disorders like Down syndrome and familial dysautonomia (FD). Congenital Insensitivity to Pain with Anhidrosis (CIPA) is associated with decreased sensation and autonomic dysfunction, somehow similar to FD. In this study we examined the effect of CIPA on pre and post-natal enamel mineralization of 9 primary extracted teeth and match-paired normal teeth. Slices of 200 microns were performed, the neonatal line was marked using a light microscope and pre and post natal enamel mineral content was determined using SEM-EDS program. The results showed marked disturbance of mineralization in relation to the severity of clinical signs in the CIPA children. In the severe case pre and post natal enamel mineralization was affected, in the moderate case only the prenatal enamel was affected while in the mild cases there was no differences in mineralization in comparison with normal teeth. Post natal traumatic lines were observed in 4 out of 9 of CIPA teeth in the children with severe and moderate clinical signs. In comparison to FD, in CIPA the effect on mineralization was observed in both pre and post natal enamel and related to the severity of clinical signs, and less post-traumatic lines were observed.

Keywords: *CIPA; Enamel; Mineralization; Post-Natal Traumatic Lines*

Introduction

Differentiation of human primary teeth formation starts during the 6 - 8th week *in utero* [1,2], and is a strictly ordered hierarchical process, with limited duration. It provides data on different developmental traumatic events within an individual, due to the consecutive proceeding along the tooth row [1-3].

It is well established that severe systemic stress occurrences, during teeth development, leave permanent imprints on the teeth, and their location reflects the timing and duration of such events [4]. Once these teeth are shed, they can be studied without direct involvement of the subject and used to reconstruct the past developmental history of an individual during gestation and infancy [5,6]. By examination of such teeth, the developmental defects may elucidate prenatal stress and help to identify those that occurred before and during infancy.

Bio-mineralisation of tooth enamel takes place in a highly structured organic matrix, laid down by the ameloblasts. It is divided into three stages, classified as forming, maturing and mature, during which mineral content increases and organic content decreases. Impaired ameloblasts function will affect the quantity and the integrity of the organic matrix. Thereby, it will affect the orientation and growth of the apatite crystals and subsequently degrade and remove the organic matrix.

Congenital Insensitivity to Pain with Anhidrosis (CIPA- OMIM#256800), also known as Hereditary Sensory and Autonomic Neuropathy (HSAN) type IV, is the most common and widely recognized of the congenital sensory neuropathies. It is an autosomal recessive disorder of the nervous system that inhibits pain sensation (causes insensitivity to pain), temperature changes (temperature sensing defects\ defective thermoregulation), or any sensory nerve-related feeling (except pressure). Anhidrosis means that the body is not sweating (fails\inability to sweat) [7].

It is characterized by recurrent episodic fevers, anhidrosis, absent reaction to noxious stimuli, self-mutilating behavior and mental retardation. In younger children, self-mutilation such as tongue or finger biting is very common and requires preventive dental extractions. In older children, osteomyelitis and bone/joint deformities require frequent surgical procedures including amputations [7,8]. Special training programs to prevent self-mutilation and accidental injuries are necessary but may be hampered by patients' cognitive deficiencies [9].

One of 5 patients (20%) succumbs to hyperpyrexia, mostly before age 3 years. Most children are mentally retarded, with IQs varying from 41 to 78, the majority being in the 60s. Some of them demonstrate recurrent episodes of unexplained high fever without sweating, repeated traumatic and thermal injuries, and self-mutilating behavior [9-11].

In a group of 15 Israeli-Bedouin children with CIPA, in which the disorder has a relatively high prevalence, Yagev and co-workers [12] found that all had absent corneal sensation, which led to corneal opacities in 10 (67%). In 7 children they found active corneal ulcers; two children had bilateral ulcers, of which 3 were recurrent and characterized by very poor healing.

In two earlier research studies, Zilberman and associates demonstrated that identification of the onset of developmental insults through examination of exfoliated deciduous teeth may help to differentiate between intra-uterine and perinatal factors that result in developmental disturbances during infancy and childhood [5-6]. This study was design to examine the effect of this congenital be "sensory neuropathy on enamel development in primary teeth. The neonatal line was used to distinguish between pre and postnatal mineralization of the enamel.

Materials and Methods

The study was carried out on ground sections from 18 primary teeth- nine extracted teeth from 7 children diagnosed with CIPA (a child with severe clinical signs-two teeth, one child with moderate clinical signs-two teeth and 5 children with mild clinical signs-one tooth from each) and nine match-paired control teeth normally exfoliated from healthy children. The CIPA patients were treated at the Oral Surgery Clinic, Ben- Gurion University of the Negev, by one of the authors (LB). The teeth were kept in 10% neutral buffered formalin solution. The analyses were performed on ground sections prepared from the teeth using a Beuhler isomet diamond wafering blade saw. The teeth were embedded in acrylate and a 200 microns thick section was obtained from each tooth after sectioning and polishing. The section was cut bucco-lingually parallel to the long axis of the tooth. The anterior teeth were sectioned in the middle of the crown mesio-distally, the canine slice included the cusp tip and on the molars the sectioning was performed on a line connecting the mesiobuccal and mesiolingual cusps. The slices were photographed using a light microscope (Best Scope) at x20 enlargement and the location of the neonatal line was marked on each slice. The chemical analyses were carried on two squares of 0.1 sqmm on each slice, one on prenatal enamel and one on post natal enamel (Figure 1). By using scanning electron microscopy (SEM, Quanta 200, Oregon, USA) in a low vacuum mode in conjugation with an energy dispersive x-ray spectrometer (EDS), the main and trace elements of the enamel were identified. On each square more than 3000 measurements were performed. The main elements were calcium, phosphate, oxygen and carbon. The trace elements included magnesium, silica, sodium, alumina and chlorine. The results are in mol.wt - &mean and SD.

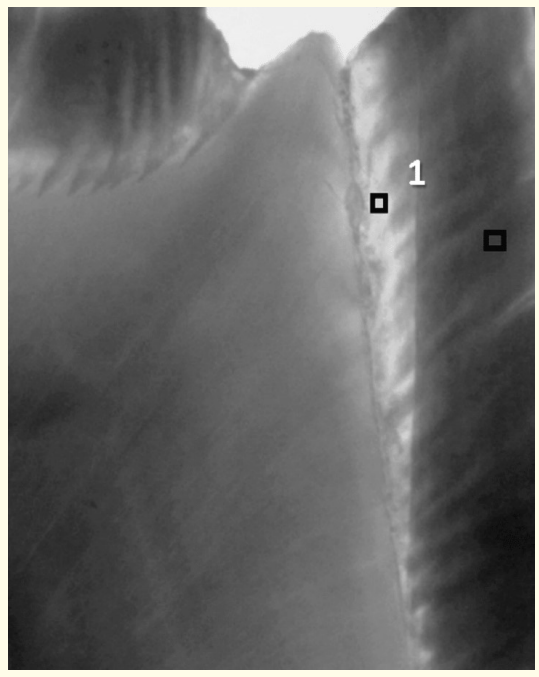

Figure 1: *Location of chemical analysis on pre-natal and post-natal enamel.*

Note: 1: Neo-natal line.

Results

Table 1 shows the chemical composition of the CIPA teeth and the match-paired normal teeth. Three different groups can be defined related to the chemical composition of the enamel in CIPA teeth. The two teeth from the severely affected CIPA child (primary lower canine and first molar) showed marked disturbances of mineralization both in the pre and postnatal enamel- the amount of calcium and phosphate was reduced by 40% compared to normal, and the concentration of silica and carbon was very high. The two teeth from the moderate affected child (two primary lower molars) showed mineralisation disturbance mainly in the prenatal enamel- 20% reduction in the concentration of calcium and phosphate and high carbon content. The postnatal enamel showed similar results as the control teeth. The five remaining teeth from mild affected CIPA patients showed similar ion content as the control teeth. Post natal traumatic lines were observed in four CIPA teeth, the teeth from the children with severe and moderate clinical signs (Figure 2). No postnatal traumatic lines were observed in normal teeth.

	Tooth	Enamel	Ca	P	O	C	Si	Mg	Al	Na	Cl
Normal	Lower Dc	Prenatal Postnatal	32.24 (0.20) 29.38 (0.20)	16.07 (0.12) 16.03 (0.12)	43.13 (0.39) 46.80 (0.41)	6.29 (0.19) 5.62 (0.18)	1.25 (0.06) 1.24 (0.06)	0.15 (0.02) 0.12 (0.02)	0.07 (0.02)	0.88 (0.06) 0.55 (0.06)	0.20 (0.03)
CIPA	Lower Dc	Prenatal Postnatal	19.04 (0.17) 18.69 (0.18)	10.93 (0.13) 11.06 (0.14)	45.06 (0.41) 41.83 (0.40)	14.66 (0.41) 16.39 (0.46)	9.35 (0.10) 10.91 (0.11)	0.19 (0.03) 0.21 (0.03)		0.78 (0.08) 0.68 (0.04)	0.23 (0.03)
Normal	Lower Dm1	Prenatal Postnatal	35.02 (0.22) 35.84 (0.23)	16.74 (0.13) 17.19 (0.11)	40.70 (0.39) 39.88 (0.38)	5.70 (0.18) 5.74 (0.18)	0.60 (0.06) 0.21 (0.03)	0.14 (0.02) 0.14 (0.02)	0.06 (0.02)	0.83 (0.07) 0.62 (0.07)	0.21 (0.03) 0.38 (0.03)
CIPA	Lower Dm1	Prenatal Postnatal	21.97 (0.18) 14.87 (0.17)	11.95 (0.13) 10.57 (0.13)	43.52 (0.41) 50.38 (0.46)	14.29 (0.40) 17.41 (0.48)	7.29 (0.09) 5.77 (0.10)	0.20 (0.02) 0.16 (0.03)		0.78 (0.07) 0.84 (0.09)	

a. Severely affected CIPA child.

	Tooth	Enamel	Ca	P	O	C	Si	Mg	Al	Na	Cl
Normal	Lower Dm2	Prenatal Postnatal	35.59 (0.23) 35.86 (0.20)	16.99 (0.11) 17.38 (0.11)	40.77 (0.39) 41.00 (0.37)	4.93 (0.16) 4.65 (0.15)	0.11 (0.02) 0.16 (0.02)	0.26 (0.02) 0.22 (0.02)		1.08 (0.07) 0.99 (0.06)	
CIPA	Lower Dm2	Prenatal Postnatal	28.71 (0.18) 34.49 (0.22)	13.16 (0.11) 16.46 (0.12)	36.25 (0.38) 39.51 (0.38)	9.84 (0.30) 7.03 (0.21)	1.12 (0.05) 1.06 (0.06)	0.19 (0.04) 0.21 (0.02)	0.07 (0.02)	0.62 (0.06) 0.90 (0.04)	0.28 (0.03)
Normal	Lower Dm2	Prenatal Postnatal	35.66 (0.22) 36.20 (0.22)	16.90 (0.11) 17.03 (0.11)	39.60 (0.38) 39.37 (0.38)	6.39 (0.20) 5.94 (0.19)	0.26 (0.02) 0.18 (0.02)	0.19 (0.02) 0.19 (0.02)		0.80 (0.07) 0.70 (0.07)	0.21 (0.03) 0.38 (0.03)
CIPA	Lower Dm2	Prenatal Postnatal	29.88 (0.21) 33.16 (0.21)	14.50 (0.11) 16.00 (0.11)	36.36 (0.37) 39.86 (0.38)	17.86 (0.48) 9.55 (0.27)	0.04 (0.02) 0.08 (0.02)	0.16 (0.02) 0.23 (0.02)	0.06 (0.02) 0.08 (0.02)	0.86 (0.07) 0.72 (0.07)	0.28 (0.03) 0.33 (0.03)

b. Moderate affected CIPA child.

	Tooth	Enamel	Ca	P	O	C	Si	Mg	Al	Na	Cl
Normal	Upper Di1	Prenatal Postnatal	31.62 (0.21) 33.64 (0.21)	17.82 (0.12) 16.91 (0.11)	43.13 (0.40) 40.95 (0.39)	6.11 (0.19) 6.92 (0.21)	0.20 (0.02)	0.25 (0.02) 0.16 (0.02)		0.88 (0.07) 0.99 (0.07)	0.43 (0.06)
CIPA	Upper Di1	Prenatal Postnatal	33.63 (0.22) 34.12 (0.22)	16.59 (0.11) 17.09 (0.11)	40.95 (0.39) 39.17 (0.38)	7.63 (0.23) 8.18 (0.24)		0.21 (0.02) 0.23 (0.02)		0.69 (0.07) 0.80 (0.04)	0.30 (0.03) 0.41 (0.06)
Normal	Upper Di2	Prenatal Postnatal	35.03 (0.22) 34.69 (0.22)	16.77 (0.12) 16.81 (0.12)	40.89 (0.38) 40.36 (0.38)	5.47 (0.17) 5.95 (0.19)	0.52 (0.06) 0.80 (0.03)	0.21 (0.02) 0.16 (0.02)	0.08 (0.02)	0.89 (0.04) 0.69 (0.04)	0.24 (0.03) 0.47 (0.03)
CIPA	Upper Di2	Prenatal Postnatal	35.54 (0.22) 34.93 (0.22)	17.17 (0.11) 17.31 (0.11)	38.51 (0.38) 38.80 (0.38)	7.42 (0.22) 7.42 (0.22)	 0.17 (0.02)	0.20 (0.02) 0.24 (0.04)		0.94 (0.07) 0.67 (0.07)	0.21 (0.03) 0.46 (0.030
Normal	Lower Di2	Prenatal Postnatal	35.33 (0.22) 36.88 (0.23)	16.81 (0.11) 16.56 (0.11)	39.39 (0.38) 37.69 (0.38)	6.82 (0.21) 7.42 (0.22)	0.33 (0.03) 0.21 (0.03)	0.14 (0.02) 0.17 (0.02	0.06 (0.02) 0.06 (0.02)	0.66 (0.07) 0.84 (0.07)	0.46 (0.03) 0.17 (0.03)
CIPA	Lower Di2	Prenatal Postnatal	34.83 (0.22) 33.46 (0.21)	17.07 (0.13) 16.60 (0.13)	39.29 (0.38) 39.69 (0.38)	6.84 (0.21) 7.68 (0.23)	0.69 (0.06) 1.16 (0.06)	0.22 (0.02) 0.26 (0.03)		0.95 (0.07) 0.91 (0.07)	0.23 (0.03)
Normal	Lower Dm1	Prenatal Postnatal	35.06 (0.220 34.63 (0.22)	16.48 (0.13) 16.92 (0.13)	39.40 (0.38) 39.98 (0.38)	7.08 (0.22) 6.74 (0.21)	0.83 (0.06) 0.47 (0.06)	0.13 (0.02) 0.16 (0.02)	0.07 (0.02) 0.03 (0.02)	0.81 (0.07) 0.70 (0.04)	0.14 (0.03) 0.38 (0.03)
CIPA	Lower Dm1	Prenatal Postnatal	35.41 (0.22) 33.95 (0.22)	17.17 (0.11) 16.74 (0.11)	39.57 (0.38) 39.84 (0.38)	6.54 (0.20) 8.15 (0.24)	0.23 (0.03) 0.14 (0.02)	0.18 (0.02) 0.26 (0.02)		0.60 (0.07) 0.92 (0.07)	0.30 (0.03)
Normal	Upper Dm1	Prenatal Postnatal	34.53 (0.22) 35.00 (0.22)	16.82 (0.11) 16.94 (0.11)	40.21 (0.38) 39.29 (0.38)	6.92 (0.21) 7.28 (0.22)	0.32 (0.03) 0.29 (0.03)	0.17 (0.02) 0.18 (0.02)		0.85 (0.07) 0.72 (0.07)	0.17 (0.03) 0.30 (0.03)
CIPA	Upper Dm1	Prenatal Postnatal	31.61 (0.21) 33.06 (0.21)	17.11 (0.13) 16.30 (0.13)	41.48 (0.39) 40.83 (0.38)	6.76 (0.21) 6.83 (0.21)	1.67 (0.07) 1.49 (0.07)	0.18 (0.02) 0.21 (0.02)	0.03 (0.02) 0.05 (0.02)	0.79 (0.07) 0.98 (0.07)	0.38 (0.03) 0.25 (0.03)

c. Mild affected CIPA children.

Table 1: *Chemical composition of CIPA primary teeth and match-paired normal teeth.*

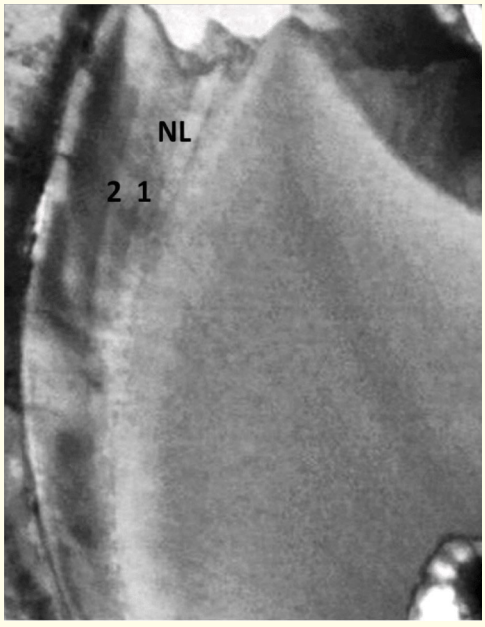

Figure 2: *Two postnatal traumatic lines on enamel of CIPA primary second molar.*

Note: NL: Neo-natal line; 1,2: Post natal traumatic lines

Discussion

CIPA or HSAN IV is caused by mutations in the NTRK1 (TRKA) gene that is located on chromosome 1 (1q21-q22). This gene encodes for neurotropic tyrosine kinase receptor type 1 that is autophosphorylated in response to NGF (nerve growth factor) [13]. As a result of loss of function mutations, signal transduction at the NGF receptor is impeded and NGF dependent neurons, the small sensory and sympathetic neurons fail to survive. For the HSAN disorder penetrance is complete but there can be marked variability in expression. Like Familial Dysautonomia (FD), CIPA is associated with decreased sensation and autonomic dysfunction. It may be confused with FD in the neonatal period but the differences become much clearer with time, as the characteristic anhidrosis causes cutaneous changes and the sensory insensitivity is much more profound, resulting in self-mutilation, auto-amputation and corneal scaring [14]. Neuropathological studies have demonstrated decreased neuronal populations. Sural nerve biopsies demonstrate presence of myelinated nerve fibers, and absent unmyelinated in the dorsal root ganglion. Neuronal atrophy and degeneration predominantly affecting peripheral sensory neurons have been reported [15]. The dorsal root ganglia develop in the embryo from neural crest cells, not neural tube. The neural crest cells are also essential to tooth formation [4]. Oral manifestation of CIPA children include premature tooth loss, lacerations and ulcerations of oral soft tissues, limited ability to open the mouth due to intra-oral scarring, severe dental attrition and dental luxation and a high incidence of dental caries [16]. Ultrastructural and morphometric studies of one shed primary tooth revealed dental abnormalities including hypomineralisation, dentin hypoplasia, cementogenesis defects and a dysplastic periodontal ligament [17].

The results of this study showed marked disturbances in mineralisation of primary teeth related to the variability of expression of CIPA clinical signs. In the severe case a marked reduction in calcium and phosphate was observed in both prenatal and postnatal enamel together with an increase in carbon and silica ions. In the moderate case only in the prenatal enamel a marked reduction of calcium and phosphate was observed, together with an increase in carbon ion while the post natal enamel was almost similar to normal. In the mild

cases the ion concentration in both prenatal and postnatal enamel was similar to normal teeth. In comparison to the dental mineralization of familial dysautonomia, where only the prenatal enamel was significantly affected [6], in CIPA children the effect on enamel mineralization was related to the severity of the symptoms. Postnatal traumatic lines were observed in all FD primary teeth compared to only 45% in the CIPA teeth and the number of traumatic lines was higher than in the CIPA primary teeth, implicating that dental mutilation during post natal enamel development was significantly higher in FD children.

Conclusions

1. Mineralization of primary teeth in CIPA teeth was affected in relation to severity of clinical signs.
2. Prenatal enamel was affected in both severe and moderate cases.
3. Post natal enamel was affected only in the severe case.
4. Post natal traumatic lines were observed in both severe and moderate affected cases.
5. CIPA, a degenerative neural disorder affects primary teeth mineralization due to the abnormality of neural crest cells, affect teeth formation and development.

Authors' Contribution to the Manuscript

Uri Zilberman, Arwa Gera and Eliyahu Mass contributed to the conception and design of the study, collection of normal teeth, analysis, interpretation of data and drafting the article. Lipa Bodner collected the teeth of CIPA and made substantial contribution to final approval of the version to be submitted, together with all other authors. All authors have read and approved the final article.

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