The Effect of Multiple Sulfatase Deficiency (MSD) on Dental Development: Can We Use the Teeth as an Early Diagnostic Tool?

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Abstract *Background:* Multiple sulfatase deficiency (MSD) is a rare autosomal recessive inborn error of metabolism due to reduced catalytic activity of the different sulfatase. Affected individuals show neurologic deterioration with mental retardation, skeletal anomalies, organomegaly, and skin changes as in X-linked ichthyosis. The only organ that was not examined in MSD patients is the dentition.

Objectives: To evaluate the effect of the metabolic error on dental development in a patient with the intermediate severe late-infantile form of MSD (S155P).

Methods: Histological and chemical study were performed on three deciduous and five permanent teeth from MSD patient and pair-matched normal patients.

Results: Tooth germ size and enamel thickness were reduced in both deciduous and permanent MSD teeth, and the scalloping feature of the DEJ was missing in MSD teeth causing enamel to break off from the dentin. The mineral components in the enamel and dentin were different.

Conclusions: The metabolic error insults the teeth in the stage of organogenesis in both the deciduous and permanent dentition. The end result is teeth with very sharp cusp tips, thin hypomineralized enamel, and exposed dentin due to the break off of enamel. These findings are different from all other types of MPS syndromes.

Clinically the phenotype of intermediate severe late-infantile form of MSD appeared during the third year of life. In children of parents that are carriers, we can diagnose the disease as early as birth using X-ray radiograph of the anterior upper region or as early as 6–8 months when the first deciduous tooth erupt and consider very early treatment to ameliorate the symptoms.

Introduction

Multiple sulfatase deficiency (MSD, MIM 272200) is a rare autosomal recessive inborn error of metabolism. This error results in tissue accumulation of sulfatides, sulfated glycosaminoglycans, sphingolipids, and steroid sulfates (Hopwood and Ballabio 2001). The enzymatic defect affects the whole family of sulfatase enzymes. MSD is caused by mutations in the sulfatase-modifying factor 1 gene (SUMF1), located on 3p26.1 (Schlotawa et al. 2008), encoding the formylglycine-generating enzyme (FGE). The clinical presentation includes symptoms of the different sulfatase deficiencies. The abnormal activity leads to an accumulation of complex sulfatase esters and eventually to variable clinical phenotypes. Affected individuals show neurologic deterioration with mental retardation, skeletal anomalies, organomegaly, and skin changes as in X-linked ichthyosis. Different types of MSD can be distinguished according to the age of onset: neonatal, late infantile, and juvenile (Hopwood and Ballabio 2001). Neonatal is the most severe form with a broad range of mucopolysaccharidosis-like clinical symptoms and death within the first year of life. Late-infantile MSD, which includes the majority of cases, resembles late-infantile metachromatic leukodystrophy with progressive loss of mental and motor abilities, combined with other symptoms of single sulfatase defi-

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ciencies like dysmorphism, skeletal changes, and ichthyosis. Attenuate form of late-infantile MSD with onset of symptoms in late childhood, beyond the second year of life, and slower progression was also documented (Schlotawa et al. 2008). Rare cases of juvenile-onset MSD have been reported with onset of symptoms in late childhood and slower progression (Blanco-Aguirre et al. 2001).

Clear genotype/phenotype correlation among patients with MSD was determined (Schlotawa et al. 2011). The patient with neonatal onset had marked impairments in both SUMF1 stability and almost undetectable enzyme activity. Patient with mild late-infantile onset were homozygous for a missense mutation (G263V; 607939.0018), which showed the highest residual enzymatic activity among the studied variants. Patients with the intermediate severe late-infantile form had mutations that compromised stability and caused low levels of residual enzyme activity (e.g., S155P; 607939.0010).

Despite thorough documented insult of various organs in MSD patients, there is lack of reports on the tooth as insulted organ. The teeth offer an excellent model with which to reconstruct the timing and progress of growth insults. Their development is a strictly ordered hierarchical process correlated with chronological age and begins in the 6th to 8th week in utero (Kraus and Jordan 1965; ten Cate 1998). Crown side is determined by two phases. The early stage is defined by the dentin-enamel junction (DEJ) that denotes the tooth germ. The final size is defined by the thickness of the enamel shell. Developmental insults leave a permanent imprint, mainly in enamel, whose onset and severity can be identified in fully formed teeth. Hereditary disorders were shown to affect both apposition and mineralization of enamel in deciduous and permanent teeth (Keinan et al. 2006, 2007).

The aim of this study is to examine deciduous and permanent tooth development in a girl diagnosed with intermediate severe late-infantile form of MSD.

Case Presentation

OM, a firstborn 3-year-old girl, showed progressive neurologic and motor deterioration that started 12 months earlier. Due to her symptoms, she was evaluated by extensive biochemical studies that showed low levels of the enzymes aryl sulfatase A as in metachromatic leukodystrophy, alpha-iduronate sulfate sulfatase as in Hunter disease, and heparin sulfaminidase as in Sanfilippo A syndrome. A high level of mucopolysaccharides and sulfatide were found in the urine. Based on these results, a diagnosis of MSD was established and genetic analyses were performed. The girl was homozygous to S155P mutation and her parents were found to be carriers. At the age of 9 years, she was referred to the pediatric dental clinic at Barzilai Medical University Center, Ashkelon,

Israel, due to suspected pain from the teeth and self-mutilation of the inside of both cheeks. The girl was under medical surveillance and treated for grand mal epilepsy, tetraparesis, gastroesophageal reflux, chronic constipation, and mild ichthyosis. She was hospitalized several times due to aspiration pneumonia. The oral and dental examination revealed anterior open bite (Fig. 1a), deciduous and permanent teeth with very thin enamel on the buccal and lingual surfaces and missing enamel on the occlusal surface, and dark discoloration of dentin. The upper anterior X-ray showed upper incisors with large pulps and hypocalcified enamel in comparison with normal (Fig. 1b, c). The bite wing radiograph (Fig. 1d, e) showed very thin hypocalcified enamel on the occlusal surfaces of permanent molars and very high mesial pulp horns, in comparison with normal.

The dental treatment included extraction of 12 deciduous teeth (all canines, first and second molars) and four permanent first molars. During the following 4 years, additional 12 permanent teeth were extracted due to self-mutilation caused by the sharp edges of the exposed dentin cusps (premolars and molars) and malposition that interfered with occlusion and lip closure (upper lateral incisors and lower canines). Eventually the girl died at 13 years of age due to complicated aspiration pneumonia.

Materials and Methods

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation of Barzilai Medical Center, Ashkelon, Israel, and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from the patient's parents for being included in the study.

Teeth Preparation

Three deciduous (upper canine, #53, and upper and lower second molars #65,85) and five permanent teeth (upper second incisor, #12; first premolar, #14; and molar, #26, and lower second premolar, #35, and molar #37) from MSD and pair-matched normal patients were embedded in epoxy (Epofix Kit, Struers) and sliced buccolingual along the long axis of the tooth. The anterior teeth were sliced through the middle of the crown, the premolars were sliced on a line connecting the buccal and lingual cusps, and the molars were sliced on a line connecting mesiobuccal and mesiolingual cusps using a wafer blade (Isomet 1000, Buehler).

Measurements Performed

- a. The slice surfaces were polished and photographed using a light microscope at a 10× magnification. The

Fig. 1 Clinical and radiographic view of MSD dentition. *Note:* (a) Anterior view of MO. Note the yellowish color of teeth due to thin and hypomineralized enamel. (b) X-ray of MO upper anterior teeth. Note altered morphology and lack of enamel. (c) X-ray of normal

upper anterior teeth. (d) Left bitewing of MO. Note very thin radiolucent enamel. (e) Left bitewing of normal teeth. Note thick radiopaque enamel. (f) SEM picture of MO premolar cusp. Note the break off of enamel from the dentin

Fig. 2 The measurements performed on each tooth. *Note:* (1) Enamel width, (2) dentin cusp height, (3) maximal tooth germ width, (4) crown width at CEJ

Fig. 3 DEJ structure. *Note:* MSD relatively straight junction (*right*) vs. scalloped normal DEJ (*left*)

enamel width, maximal dentin width, tooth width at the CEJ, enamel thickness, and crown dentin height were measured (Fig. 2), using a digital caliber.

- b. The teeth surfaces were polished and analyzed using SEM under high vacuum mode and several observations were performed: the surface of the DEJ was observed at the enlargement of 200 in order to determine the degree of scalloping of the junction (Fig. 3), and the enamel and dentin chemical components were analyzed using the ESD program.

Results

The results of the measurements of tooth size are reported in Table 1. The buccolingual tooth germ size as determined

by the DEJ was reduced in MSD teeth. Except tooth #14, the width at the CEJ in MSD teeth was reduced by 10–30%, less in anterior teeth and mainly in molars. The most striking finding was the width of the enamel. In MSD deciduous teeth, the enamel width was constant at 0.3 mm, and in permanent teeth it was constant at 0.4 mm on all surfaces. In normal deciduous teeth, the enamel width was around 0.9 mm in anterior tooth and 1.2 mm in molar, while in permanent teeth the enamel width was 1.2 mm in anterior tooth and 1.8–2.1 mm in posterior teeth.

The DEJ showed a smoother line in all MSD teeth compared to normal.

Table 2 shows the enamel and dentin chemical content of MSD teeth compared to normal. The main differences were found in the residual minerals. Except tooth #85, MSD teeth showed lower content of magnesium, silica was found only in MSD teeth, and chlorine content was

Table 1 Measurements of tooth components in mm

Tooth	Status	Width at DEJ	Max dentin width	Max dentin height	Enamel width
Upper deciduous canine #53	MSD	5.3	6.3		0.3
	Normal	6.5	6.7		0.9
Lower second deciduous molar #85	MSD	6.8	7.7	5.8	0.3
	Normal	9.2	10.0	5.7	1.2
Upper permanent second incisor #12	MSD	7.0	7.0	11.7	0.4
	Normal	7.5	7.3	11.8	1.1
Upper permanent first premolar #14	MSD	10.1	10.2	9.3	0.4
	Normal	10.1	10.4	10.2	1.8
Upper permanent first molar #26	MSD	11.5	12.1	6.8	0.4
	Normal	13.2	12.1	7.5	1.9
Lower permanent second premolar #35	MSD	8.6	9.0	7.0	0.4
	Normal	11.3	11.0	6.9	1.8
Lower permanent second molar #37	MSD	9.8	10.0	5.9	0.4
	Normal	11.4	11.5	6.2	2.1

Note: MSD multiple sulfatase deficiency, DEJ dentino-enamel junction

Table 2 Enamel and dentin components in mw%

T		C	C	O	O	P	P	Ca	Ca	Ca/P	Mg	Mg	Si	Si	N
		E	D	E	D	E	D	E	D	E	E	D	E	D	D
#53	MSD	7.84	13.12	39.73	39.25	16.51	11.56	33.99	23.32	2.06	0.13	0.25	0.65	0.25	11.41
	norm	7.00	12.72	39.94	36.66	17.55	12.51	35.96	25.08	2.04	0.24	0.57			11.93
#65	MSD	5.17	10.78	38.66	38.92	17.69	11.98	36.69	24.44	2.07	0.18	0.47	0.40	0.28	12.63
	norm	6.23	10.97	39.94	38.45	17.21	12.78	35.31	25.30	2.05	0.24	0.50			11.48
#85	MSD	6.34	12.42	41.14	39.36	16.90	11.63	36.93	22.72	2.18	0.28	0.39	0.46	0.50	12.51
	norm	6.40	12.53	37.58	36.25	17.69	12.45	37.15	25.84	2.10	0.25	0.61			11.72
#12	MSD	8.04	12.98	38.55	37.72	16.83	11.31	34.75	24.00	2.07		0.11	0.80	0.17	10.34
	norm	7.39	13.51	38.69	37.12	17.24	11.83	35.41	24.38	2.05	0.22	0.48			10.52
#14	MSD	7.19	11.58	38.35	37.16	16.91	12.48	35.43	25.56	2.09	0.16	0.47	0.87	0.10	12.15
	norm	7.30	14.47	37.72	35.32	17.75	13.03	36.00	27.03	2.03	0.28	0.65			08.77
#26	MSD	6.49	12.70	39.10	38.49	17.21	11.57	35.90	23.30	2.09	0.15	0.49	0.10		12.91
	norm	6.70	12.73	38.55	37.21	17.30	12.19	36.06	25.02	2.08	0.22	0.58			11.70
#35	MSD	8.98	14.95	34.20	34.05	17.74	12.51	37.52	25.54	2.11	0.14	0.36	0.26	0.23	11.83
	norm	5.93	11.95	40.65	37.77	17.12	12.19	35.18	24.62	2.05	0.24	0.57			12.33
#37	MSD	7.59	15.88	37.48	36.68	17.31	10.63	36.18	21.84	2.09	0.12	0.15	0.25		14.40
	norm	6.14	12.72	41.08	36.66	16.89	12.51	34.66	25.08	2.05	0.28	0.57			11.93

Note: T tooth no; see description in Table 1

C carbon, O oxygen, P phosphate, Ca calcium, Ca/P calcium/phosphate, Mg magnesium, Si silica, N nitrogen, E enamel, D dentin, MSD multiple sulfatase deficiency, Norm normal

higher in MSD teeth. The hydroxyapatite formula is $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ with residual minerals and other calcium salts. The perfect ratio of Ca/P should be 1.67, but due to the additional calcium salts, the ratio is around 2 in normal teeth. The better the mineralization, the closer the ratio to

the perfect ratio. The ratio of Ca/P was higher in the MSD enamel.

The chemical content in the dentin of MSD teeth was lower in phosphate, calcium, and magnesium, except tooth #35, and contained silica in six out of eight teeth.

Discussion

We present a girl that suffered from low levels of 3 sulfatase enzymes: aryl sulfatase A, alpha-iduronate sulfate sulfatase, and heparin sulfaminidase. Lack of aryl sulfatase A enzyme leads to the accumulation of galactosyl sulfatide in the white matter of the CNS and in the peripheral nervous system. Galactosyl sulfatide and lactosyl sulfatide also accumulate within the kidney, gallbladder, and other visceral organs. The problem with reduced activity of iduronate sulfate sulfatase concerns the breakdown of glycosaminoglycans (GAGs). As a result, GAGs build up in the cells throughout the body causing distinct facial features, large heads, and CNS involvement, leading to developmental delays and neurological problems. Low levels of heparin sulfaminidase affect the breakdown of glycosaminoglycan heparin sulfate which is found in the extracellular matrix and on cell surface glycoprotein. The primary stored substrate is heparin sulfate and the clinical features of the disease are mainly neurological (Hopwood and Ballabio 2001). The results of this study showed that both enamel and dentin are affected by MSD – the enamel in thickness and mineralization (higher Ca/P ratio in MSD teeth) and the dentin at the DEJ causing a straight junction and mineralization. During amelogenesis the percentage of protein by weight dropped from 30% during the secretory stage to 2% during the early maturation stage (Fukae and Shimizu 1974). The proteinase of the metalloproteinase class present early during the secretory stage is MMP20 (Begue-Kirn et al. 1998), and the proteinase expressed from the transitional through maturation stages is KLK4 (Hu et al. 2002). In a study on MMP20 null mouse (Caterina et al. 2002; Bartlett 2013), there was evidence of improper process of amelogenin. There was altered enamel protein and enamel rod pattern and hypoplastic enamel, which induced the enamel to break off from the dentin (Caterina et al. 2002; Bartlett 2013). This process is similar to our findings in MSD (Fig. 1f).

This is the first paper to discuss the effect of intermediate severe late-infantile form of MSD on dental tissues. There are very few papers that described the effect of various types of mucopolysaccharidosis on oral and dental tissues. The case presented showed reduced activity of three enzymes. The dental findings for each of the missing enzymes were previously described. Metachromatic leukodystrophy (lack of aryl sulfatase A enzyme) caused the accumulation of abnormal quantities of sulfatides within myelinated peripheral nerves in the oral region (Gardner and Zeman 1970) and of brown metachromatic granules (sulfatides) along the nerve fibers of the dental pulp (Gardner 1967). Hunter's syndrome (lack of alpha-iduronate sulfate sulfatase enzyme) caused localized lesions in the jaws that often resemble dentigerous cysts associated

with unerupted first permanent molars. These lesions contain dense, fibrous connective tissues and large amounts of acid mucopolysaccharides. The gingival tissues are hyperplastic, hypertrophic, and enlarged while the tongue is macroglossic (Templeton-Downs et al. 1995). Enamel crystal organization is disturbed in Hunter's syndrome. The disruption affects the crystallography and nanostructure of enamel but has not yet been detected clinically (Al-Jawad et al. 2012). Sanfilippo syndrome (lack of heparin sulfaminidase enzyme) caused obliteration of pulp chambers and root canals in deciduous and permanent teeth (Mellara et al. 2012).

In other types of mucopolysaccharides accumulation, high palate, open bite, impacted teeth, changes in temporomandibular joint, taurodontism, long tooth roots, unerupted teeth, large dental follicles, and supernumerary teeth (Maroteaux–Lamy syndrome) were found (Kantaputra et al. 2014). In Morquio's disease the enamel layer was very thin but of normal radiodensity, and the radiographic appearance of the dentine, pulp chambers, and root canals was normal (Nelson and Kinirons 1988). Histological investigations have demonstrated that the enamel has increased porosity correlating to the striae of Retzius, and the hydroxyapatite crystals are smaller (Al-Jawad et al. 2012).

The observable clinical effect of the intermediate severe late-infantile form of MSD presented here started during the third year of life and was mainly in parallel to the progressive neurological and motor deterioration. The results from the dental analysis showed that the amelogenesis of the deciduous dentition was affected and showed very thin and hypomineralized enamel with a constant enamel thickness of 0.3 mm and opacity similar to dentin and reduced germ size as determined by the reduced measurements of height and width at the DEJ. In addition the scalloped feature of the DEJ was reduced and the enamel broke off from the dentin shortly after eruption, in both deciduous and permanent teeth. Each of the featured observed in MSD can be identified in other hereditary disorders of the dentition – the thin enamel can be found in hypoplastic amelogenesis imperfecta (e.g., MIM 104530 or MIM 204650), hypocalcified enamel can be found in hypocalcified amelogenesis imperfecta (e.g., MIM 130900 or MIM 104500), and enamel that breaks out of dentin with un-scalloped DEJ can be found in dentinogenesis imperfecta (e.g., MIM 125490). But all features together in both deciduous and permanent dentition are unique to this type of MSD. Since the stage of deciduous teeth amelogenesis begins at the fetus during the 6th–8th weeks in utero, these findings implicate that the effect of the disease starts during pregnancy, and one of the first organs to show deterioration is the dentition. In families of parents that are carriers for MSD, where there is a suspicion of MSD, we can diagnose

diseased children using an X-ray radiograph of the anterior region immediately after birth or at the age of 6–8 months when the first deciduous tooth erupts. The peculiar phenotype of the teeth is unique and can be easily diagnosed as MSD.

Compliance with Ethics Guidelines

Uri Zilberman and Haim Bibi declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standard of the responsible committee on human experimentation of Barzilai Medical University Center, Ashkelon, Israel, and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from the parents of the child included in this study. All details of the child included in this publication are anonymous.

Uri Zilberman performed the clinical treatment and the extractions of the teeth, the investment and the slicing of the teeth, and the SEM analysis. Uri Zilberman and Haim Bibi prepared the report and the answers to the reviewers' comments.

References

- Al-Jawad M, Addison O, Khan MA, James A, Hendriksz C (2012) *J Dent* 40:1074–1080
- Bartlett JB (2013) Dental enamel development: proteinases and their enamel matrix substrates. *ISRN Dent*. doi:10.1155/2013/684607
- Begue-Kirn C, Krebsbach PH, Bartlett JD, Butler WT (1998) Dentin sialoprotein, dentin phosphoprotein, enamelysin and ameloblastin: tooth-specific molecules that are distinctively expressed during murine dental manifestation. *Eur J Oral Sci* 106:963–970
- Blanco-Aguirre ME, Kofman-Alfaro SH, Rivera-Vega MR et al (2001) Unusual clinical presentation in two cases of multiple sulfatase deficiency. *Pediatr Dermatol* 18:388–392
- Caterina JJ, Skobe Z, Shi J et al (2002) Enamelysin (MMP20) deficient mice display an amelogenesis imperfecta phenotype. *J Biol Chem* 277:49598–49604
- Fukae M, Shimizu M (1974) Studies on the proteins of developing bovine enamel. *Arch Oral Biol* 19:381–386
- Gardner DG (1967) Pulpectomy as a diagnostic procedure in metachromatic leukodystrophy. *Oral Surg Oral Med Oral Pathol* 23:379–384
- Gardner DG, Zeman W (1970) Oral findings in metachromatic leukodystrophy. *Oral Surg Oral Med Oral Pathol* 29:431–436
- Hopwood J, Ballabio A (2001) Multiple sulfatase deficiency and the nature of the sulfatase family. In: Scriver CR, Valle D, Sly WS (eds) *The metabolic and molecular bases of inherited disease*. McGraw-Hill, New York, pp 3725–3732
- Hu JCC, Sun X, Zhang C, Liu S, Bartlett JD, Simmer JP (2002) Enamelysin and kallikrein-4 mRNA expression in developing mouse molars. *Eur J Oral Sci* 110:307–315
- Kantaputra PN, Kayserili H, Güven Y, Kantaputra W, Balci MC, Tanpaiboon P, Uttarilli A, Dalal A (2014) Oral manifestations of 17 patients affected with mucopolysaccharidosis type VI. *J Inher Metab Dis* 37:263–268
- Keinan D, Smith P, Zilberman U (2006) Microstructure and chemical composition of primary teeth in children with Down syndrome and cerebral palsy. *Arch Oral Biol* 51:836–843
- Keinan D, Smith P, Zilberman U (2007) Prenatal growth acceleration in maxilla deciduous canines of children with Down syndrome: histological and chemical composition study. *Arch Oral Biol* 52:961–966
- Kraus BS, Jordan RE (1965) *The human dentition before birth*. Lea & Febiger, Philadelphia, pp 136–138
- Mellara TS, Azevedo DT, Faria G et al (2012) Dental findings and management in a mucopolysaccharidosis type IIIB patient. *J Dent Child (Chic)* 79:176–180
- Nelson J, Kinirons M (1988) Clinical findings in 12 patients with MPS IV A (Morquio's disease). Further evidence for heterogeneity. Part II: dental findings. *Clin Genet* 33:121–125
- Schlotawa L, Steinfeld R, von Figura K, Dierks T, Gartner J (2008) Molecular analysis of SUMF1 mutation: stability and residual activity of mutant formylglycine-generating enzyme determine disease severity in multiple sulfatase deficiency. *Hum Mutation* 29:205–217
- Schlotawa L, Ennenmann EC, Radhakrishnan K et al (2011) SUMF1 mutations affecting stability and activity of formylglycine generating enzyme predict clinical outcome in multiple sulfatase deficiency. *Eur J Human Genet* 19:253–261
- Templeton-Downs A, Crisp T, Ferretti G (1995) Hunter's syndrome and oral manifestations: a review. *Pediatr Dent* 17:98–100
- ten Cate AR (1998) *Oral histology. Development, structure and function*. 5th edn. The CV Mosby Co., St. Louis, pp 73–102