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Research Article

Examination of Enzymatic Concentration of MMP20 and KLK4 in Serum and Saliva of Children Ages 0 - 5 Years

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Abstract

Purpose: To analyze the concentration of two proteases, MMP20 and KLK4 in the serum and saliva of children 0 - 5 years old, in order to correlate between the proteases concentration and MIH appearance.

Methods: 1 ml of serum was collected from 500 children hospitalized for common childhood diseases. Saliva was collected from a different sample of 100 children. The concentration of KLK4 and MMP20 was determined using the ELISA method using testing kits.

Results: The concentration of KLK4 in the serum was between 0.26 - 53.76 ng/ml (mean 8.69 ng/ml) and in the saliva between 0.1 - 27.9 ng/ml (mean 1.01 ng/ml). The concentration of MMP20 in the serum was between 0.30 - 198.62 ng/ml (mean 31.62 ng/ml) and in the saliva between 0.32 - 29.81 ng/ml (mean 3.72 ng/ml). In the serum and saliva, girls showed statistically higher concentration of MMP20 in comparison with boys. In the saliva only, girls showed higher concentrations of KLK4 with statistical significance. The concentration of MMP20 in the young group < 24 months was reduced.

Conclusion: The reduced concentration of MMP20 in boys age < 24 months may explain the clinical finding that only two thirds of the crown is affected by MIH. The finding that the concentration of MMP20 in girls is higher compared to boys may explain the lower prevalence of MIH in girls, in the same regional community.

Keywords: *Amelogenesis; MMP20; KLK4; Amelogenin; Enamelin*

Introduction

Enamel is a hard nanocomposite bioceramic with significant resilience that protects the mammalian tooth from external physical and chemical damages for a long period of time. The enamel layer is unique because it is the only epithelial derived calcified tissue in vertebrates and is the hardest substance in the body. Enamel hardness is a function of its high mineral content and fully formed enamel contains very little protein (less than 1% organic material) [1]. Enamel development (amelogenesis) has four stages, defined by the morphology and function of the ameloblasts: pre-secretory, secretory, transition and maturation. During the pre-secretory stage the ameloblasts develop an extensive protein synthetic apparatus and prepare to secrete the organic matrix of enamel. Secretory stage enamel is protein rich and has a soft consistency. The ameloblasts starts secreting large amounts of enamel matrix proteins and elaborate and organize the entire enamel thickness. In association with newly secreted proteins, long thin mineral ribbons form. The ameloblasts secrete several proteins into the enamel ma-

trix: amelogenin (80 - 90% of the organic matter), ameloblastin (only 5%) and enamelin (3 - 5%). During the maturation stage the previously secreted matrix proteins are removed and the crystallites expand in volume [2,3]. During enamel formation almost all of the protein supporting mineral formation is removed by two proteases: matrix metalloproteinase 20 (MMP20) and kallikrein 4 (KLK4) [4]. During the secretory stage MMP20 is secreted and its role is to cleave the most abundant enamel matrix protein amelogenin [5,6]. KLK4 is secreted during the transition and maturation stages of amelogenesis, immediately preceding the point where the quantity of enamel proteins in the matrix drops. Its role is to cleave the residual enamel matrix, facilitating its removal from the enamel layer and allowing the crystals to grow in width and thickness until adjacent crystals contact. This activity is essential for hardening of the enamel layer [7].

Inherited enamel defects are designated as Amelogenesis Imperfecta (AI). Three main types are defined: Hypoplastic or thin enamel, hypomaturation enamel where the enamel is soft due to failure to remove enamel matrix proteins and hypocalcified enamel that represent a disturbance in both early and late enamel development and the enamel is soft, rough and rapidly lost by attrition. Mutations in AMELX (the amelogenin gene located on chromosome Xp22.3-p22.1), ENAM (the enamelin gene located on chromosome 4q21), MMP20 and KLK4 genes are associated with specific types of AI [8]. Human MMP20 is expressed from a gene on chromosome 11q22-23 and KLK4 gene is located on chromosome 19q13. Human mutations in genes coding for the enamel proteinases (MMP20 and KLK4) cause variable degrees of hypomineralization with a normal thickness of enamel [9,10]. KLK4 mutation caused hypomineralised enamel with less calcium and phosphorous and more nitrogen in the inner enamel, causing softer enamel in comparison to outer enamel [11]. Homozygous missense mutation of MMP20 with no functional activity caused hypomaturation AI with dark brown discoloration while compound heterozygous MMP20 mutation with reduced functional activity showed yellowish discoloration with reduced transparency [12].

MIH (Molar Incisor Hypomineralization), a less than normal level of calcification of enamel of permanent teeth, was first renamed by Weerheijm and associates in 2001 [13]. It affects amelogenesis of 1 to 4 first permanent molars and accompanied by similar damage to the permanent incisors in the occlusal two-thirds of the crown, that occurs during the first two years of age. Clinically the hypomineralization is observed on the enamel as a yellow-brown discoloration with clear or opaque borders. The hypomineralization lesion is normally found in areas that are not normally attacked by dental caries. The enamel is softer and has increased permeability, and post eruption break down may occur. The affected enamel may break down due to normal chewing leaving the exposed dentin very sensitive to external influences that can lead to mild to severe inflammation of the pulp. The clinical significance of MIH is the sensitivity to air, cold, and heat in the permanent teeth of young children. The risk of caries lesion development within the area of affected enamel is high even in children with low risk of carious activity [14]. In first molar affected by MIH the mineral content was significantly lower. The reduction of calcium was by 35% and of phosphate by 60%. The oxygen and carbon content was increased by more than 300% [15].

Mineral density of MIH molars showed on average 19% reduction compared to sound enamel and mineral content was in average only 58% vol% mineral [16,17]. In contrast protein content was 15 - 21 folds higher in brown enamel and 8-folds in yellow and chalky enamel compared to normal enamel [18].

In other words, the very high protein content in MIH teeth may be caused by reduced activity of MMP20 and KLK4 during the mineralization period of first permanent molars and incisors. Since MIH affects only occlusal two thirds of crown formation of the molars and incisors, surfaces that are not prone to carious attack, the period for this appearance is during the first two years of life.

The hypothesis of this research is that abnormal concentrations of MMP20 during the secretory stage and of KLK4 during the transitional and maturation stages may affect enamel proteins degradation and cause the clinically observed MIH.

Aim of the Study

The final aim of the study is to correlate between MMP20 and KLK4 concentration in children 0 - 5 years of age to the prevalence of MIH in permanent teeth. The first stage, analyzed in this research, is to examine the concentration of the two proteases in the serum of children and to compare it to the concentration of these proteases in the saliva. The collection of saliva is more easy and acceptable to the children and parents, and can be performed also in dental clinics. There is a need to relate it to the concentration in

the serum to understand if it is suitable for the clinical research to follow.

Materials and Methods

Population: The research was conducted on blood samples and saliva of children who had been admitted to the children's ward at French Medical Center, Nazareth, due to common childhood illnesses. The work has been carried in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The research was approved by the Helsinki committee of the French Medical Center, Nazareth (no 0049-17-EMC). Informed consent was obtained from the parents of the children examined. The number of participants in the study was 500 children between the ages of 0 - 5 years old for the serum collection and a different group of 100 children for the saliva collection.

Criteria for participation: Children between the ages of 0 - 5 years of age that arrived to the children's ward of French Hospital due to a common childhood illness requiring a blood test analysis.

Exclusion from participation: Children whose parents refused to sign an informed consent agreement, children with chronic illness or congenital malformations.

Collection of samples: The serum was taken from tubes on hand that were primarily used for routine blood testing, after centrifugation. The saliva was collected using a salivary device from the pool under the anterior part of the tongue near the Warton's duct. 1 cc of plasma and 1 cc of saliva were transferred to a sterile, numbered tube. The number will appear on a protected list at French Hospital in Nazareth where the actual name, ID number, the name of the child's father and his age is kept, for privacy protection of the participants. The list is necessary in order to prevent repeated checks on the same child if, unfortunately, the child is hospitalized again during the research period.

Analysis procedure: The tests tubes were saved in refrigeration as required in accordance to the instructions of the testing kits until the ELISA analysis took place. The tests for enzymatic activity of KLK4 and MMP20 were performed using the ELISA method using testing kits at the biochemistry laboratory of Assaf Harofeh hospital. MMP 20 human matrix metalloproteinase-20 (MMP20) and human kallikrein-4 (KLK4) were assessed in serum and saliva samples by specific ELISA kits (BIOMATIK Cat EKC34563, EKC34330 respectively), according to the manufacturer's instructions.

The results were statistically analyzed using the SPSS program.

Results

Out of 500 children, only 478 tubes with serum were included in the research. The exclusion of 22 tubes was due to hemolytic serum. Table 1 shows the descriptive analysis of the sample. The age of the children was between 1 - 60 months. The concentration of KLK4 in the serum was between 0.26 - 53.76 ng/ml (mean 8.69 ng/ml) and in the saliva was between 0.1 - 27.9 ng/ml (mean 1.01 ng/ml). The concentration of MMP20 in the serum was between 0.30 - 198.62 ng/ml (mean 31.62 ng/ml) and in the saliva between 0.32 - 29.81 ng/ml (mean 3.72 ng/ml).

	Serum			Saliva		
	Age months	KLK ng/ml	MMP20 ng/ml	Age months	KLK ng/ml	MMP20 ng/ml
Mean	20.7	8.69	31.62	37.7	1.01	3.72
Median	16	6.25	21.57	40	0.21	1.15
Std Dev	16.97	8.55	32.29	17.9	3.46	6/01
Min	1.0	0.26	0.30	2.0	0.10	0.32
Max	60.0	53.76	198.6	60.0	27.9	29.81
N	478	478	478	100	100	100

Table 1: Descriptive analyses of the serum and saliva sample.

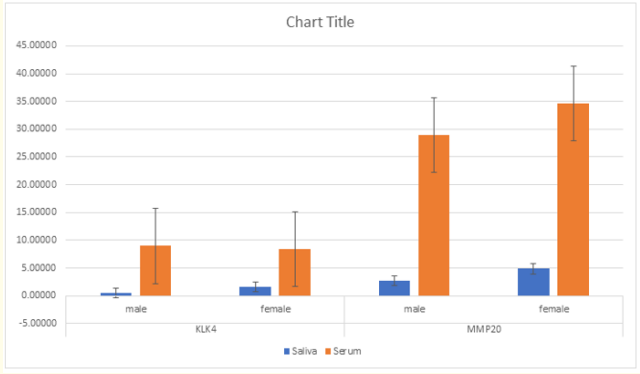
Table 2 and graph 1 shows the differences between boys and girls. In the serum the differences between boys and girls for KLK4 were not significant, but for MMP20, girls showed higher results with statistical significance (P = 0.049). In the saliva girls showed higher concentrations of both KLK4 and MMP20 but without statistical significance.

The sample was divided in 2 age groups: 0-24 months, and above 24 months. Table 3 shows the concentrations of KLK4 and MMP20 by age groups. In the serum KLK4 showed a significant decrease in the > 24 months age group (P = 0.026), while MMP20 showed an insignificant increase. In the saliva both KLK4 and MMP20 showed higher concentrations in the older group.

	Serum N	Mean ng/ml	Std Dev	Saliva N	Mean ng/ml	Std Dev
KLK4 Boys	254	8.98	8.52	53	0.50*	0.88
Girls	224	8.37	8.60	47	1.60	4.95
Total	478	8.69	8.55	100	1.01	3.46
MMP20 Boys	254	28.96*	28.93	53	2.70*	4.60
Girls	224	34.64	35.53	47	4.89	7.19
Total	478	31.62	32.28	100	3.72	6.01

Table 2: The effect of gender on KLK4 and MMP20 concentrations in serum and in saliva.

Note: *= P value < 0.05 in comparison with girls.

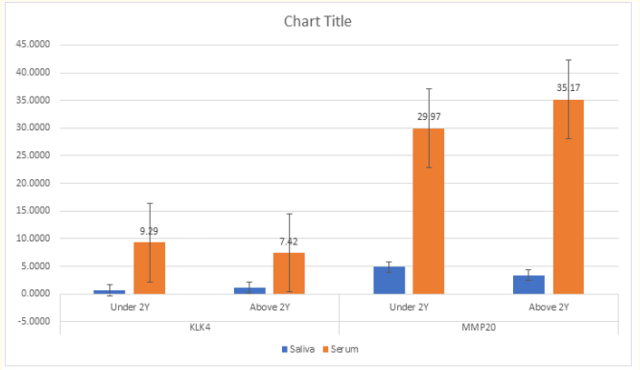


Graph 1: MMP20 and KLK4 concentrations in serum and saliva by gender.

	Age (months)	Serum N	Mean ng/ml	Std Dev	Saliva N	Mean ng/ml	Std Dev
KLK4	≤ 24	316	9.29*	8.54	25	0.65	1.01
	> 24	162	7.42	8.49	75	1.11	3.88
MMP20	≤ 24	316	29.97	29.39	25	0.30	4.84
	> 24	162	35.17	37.61	75	0.52	3.41

Table 3: Concentration of KLK4 and MMP20 by age groups in serum and in saliva.

Note: *= P value < 0.05.



Graph 2: MMP20 and KLK4 concentrations in serum and saliva by age groups.

Graph 1 shows the mean and SD of MMP20 and KLK4 in serum and saliva by gender. For MMP20, the concentration in girls was higher in both the serum and saliva but the ratio between the concentrations in serum and saliva is different. In boys the ratio between the mean concentrations of MMP20 in blood to saliva is X11 while for girls is only X7. For KLK4 the mean concentrations in serum of boys was higher than in girls while in the saliva the results showed higher concentration in girls. The ratio between serum and saliva in boys was X18 while in girls it was X5.2.

Graph 2 shows the mean and SD of MMP20 and KLK4 in serum and saliva by age groups 0 - 24 months compared to > 24 - 60 months. In serum, the concentration of MMP20 was higher in the young group, while in the saliva it was higher in the young group. The concentration of KLK4 in serum was higher in the young group while in the saliva it was higher in the old group.

Discussion

Enamelysin (MMP20) is the main enzyme expressed by the secretory stage ameloblasts and is responsible for cleavage of the matrix protein that accumulates in the enamel and is also responsible for activation of KLK4 [19,20]. KLK4 is expressed during the transitional and maturation stage ameloblasts and is responsible for protein degradation in order to allow growth of enamel crystallites in width and thickness. The digestion products are reabsorbed into ameloblasts during the maturation stage. In the absence of KLK4 expression, substantial retention of enamel proteins occurs in the enamel [21]. Specific KLK4 cleavages are responsible for inactivation of MMP20 during enamel formation [20]. Mutations in

KLK4 are associated with enamel hypomineralization and structural abnormalities [21]. In MIH teeth the protein content is higher and may reach 8 - 21 folds more, depending on the degree of hypomineralization [18]. The proteins detected in the hypomineralized region of enamel of first permanent molar affected by severe MIH included Amelogenin Y isoform, Ameloblastin, Proline rich protein and Keratins types I and II (personal data). Keratins were previously detected in enamel and are components of the organic matrix [22]. The results of this study showed that MMP20 concentration is low in children between 0 - 24 months and higher in the old group > 24 months. MMP20 is responsible for degradation of amelogenin and activation of KLK4. The reduced concentration of MMP20 during the first two years may be responsible to the finding that amelogenin Y isoform was detected in the enamel of MIH affected molars, and to the clinical finding that almost 20% of Israeli children showed MIH [23]. The increased concentration of MMP20 after 24 months may explain the clinical finding that only two-thirds of the occlusal part of the crown is affected and the gingival third is intact. In Israeli girls the concentration of MMP20 was statistically higher than in boys, a finding that correlates with the clinical finding that MIH in the age group of 6 - 10y has lower prevalence in girls in comparison to boys (16.6% in girls compared to 19.8% in boys) [23]. The reduced concentration of MMP20 in the 0 - 24 months group can also explain the low concentration of KLK4 in both age groups (MMP20 is responsible for activation of KLK4).

The second part of this research will compare clinically the appearance of MIH in the group of the children studied in this research and the concentration of MMP20 and KLK4 found in the serum. The saliva cannot be used for determination of MMP20 and KLK4 concentrations.

Conclusion

1. MMP20 and KLK4 are responsible for the cleavage of enamel proteins during the secretory, transitional and maturation stages of amelogenesis.
2. MMP20 showed significantly lower concentration in serum and saliva of boys in comparison with girls.
3. KLK4 showed similar results in serum in both genders and lower results in the saliva for boys.
4. Malfunction of MMP20 and KLK4 may affect the removal of the proteins from the enamel and cause the softer enamel observed in MIH.

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Bibliography

1. Deakins M, Volker JF. Amount of organic matter in enamel from several types of human teeth. J Dent Res. 1941;20:117-121.
2. Nanci A. Ten Cate's Oral Hystology, 9th edition. ELSEVIER St. Louis, Missouri, 2018:118-156.
3. Bartlett JD. Dental enamel development: Proteinases and their enamel matrix substrates. ISRN Dentistry. 2013:684607.
4. Simmer JP, Hu JC. Expression, structure and function of enamel proteinases. Con Tissue Res. 2002;43:441-449.
5. Llano E, Pendas AM, Knauper V, Sorsa T, Salo T, Salido E, Murphy G, Simmer JP, Bartlett JD, López-Otín C. Identification and structural and functional characterization of human enamelysin. (MMP20). Biochemistry. 1997;36:15101-15108.
6. IwataT, Yamakoshi Y, Hu JC, Ishikawa I, Bartlett JD, Krebsbach PH, Simmer JP. Processing of ameloblastin by MMP20. J Dent Res. 2007;86:153-157.
7. Lu Y, Papagerakis P, Yamakoshi Y, Hu JC, Bartlett JD, Simmer JP. Function of KLK4 and MMP20 in dental enamel formation. Biol Chem. 2008;389:695-700.
8. Wright JT, Hart PS, Aldred MJ, Seow K, Crawford PJ, Hong SP, Gibson CW, Hart TC. Relationship of phenotype and genotype in X-linked amelogenesis imperfecta. Con Tissue Res. 2003;44:72-78.
9. Hart PS, Hart TC, Michalec MD, Ryu OH, Simmons D, Hong S, Wright JT. Mutation in kallikrein 4 causes autosomal relative hypomaturation amelogenesis imperfecta. J Med Genet. 2004;41:545-549.
10. Ozdemir D, Hart PS, Ryu OH, Choi SJ, Ozdemir-Karatas M, Firatli E, Piesco N, Hart TC. MMP20 active-site mutation in hypomaturation amelogenesis imperfecta. J Dent Res. 2005;84:1031-1035.

11. Smith CEL, Kirkham J, Day PF, Soldani F, McDerra EJ, Poulter JA, Inglehearn CF, Mighell AJ, Brookes SJ. A fourth KLK4 mutation is associated with enamel hypomineralization and structural abnormalities. Front Physiol. 2017;29;8:333.

12. Kim YJ, Kang J, Seymen F, Koruyucu M, Gencay K, Shin TJ, Hyun HK, Lee ZH, Hu JC, Simmer JP, Kim JW. Analyses of MMP20 missense mutations in two families with hypomaturation amelogenesis imperfecta. Front Physiol. 2017;20;8:229.

13. Weerheijm KL, Jalevik B, Alaluusua S. Molar-incisor hypomineralization. Caries Res. 2001;35:390-391.

14. Silva MJ, Scurrah KJ, Craig JM, Manton DJ, Kilpatrick N. Etiology of molar incisor hypomineralization - A systematic review. 2016;44:342-353.

15. Zilberman U, Hassan J, Leiboviz-Haviv S. Molar incisor hypomineralization and pre-eruptive intracoronal lesions in dentistry-diagnosis and treatment planning. World J Stomatology. 2019;7:20-27.

16. Farah RA, Swain MV, Drummond BK, Cook R, Atieh M. Mineral density of hypomineralised enamel. J Dent. 2010;38:50-58.

17. Crombie FA, Manton DJ, Palamara JE, Zalizniak I, Cochrane NJ, Reynolds EC. Characterisation of developmentally hypomineralised human enamel. J Dent. 2013;41:611-618.

18. Farah RA, Monk BC, Swain MV, Drummond BK. Protein content of molar-incisor hypomineralisation enamel. J Dent. 2010b;38:591-596.

19. Nagano T, Kakegawa A, Yamakoshi Y, Tsuchiya S, Hu JC, Gomi K, Arai T, Bartlett JD, Simmer JP. Mmp20 and Klk4 cleavage site preferences for amelogenin sequences. J Dent Res. 2009;88:823-828.

20. Yamakoshi Y, Simmer JP, Bartlett JD, Karakida T, Oida S. MMP20 and KLK4 activation and inactivation interactions in vitro. Arch Oral Biol. 2013;58:1569-1577.

21. Smith CE, Richardson AS, Hu Y, Bartlett JD, Hu JC, Simmer JP. Effect of kallikrein 4 loss on enamel mineralization: comparison with mice lacking matrix metalloproteinase 20. J Biol Chem. 2011;286:18149-18160.

22. Duverger O, Beniash E, Morasso MI. Keratins as components of the enamel organic matrix. Matrix Biol. 2016;52:260-265.

23. Hassan J, Leibovitz-Haviv S, Cohen O, Zilberman U. The prevalence of Molar Incisor Hypomineralization among children in Jewish and Arab population in Israel. Journal of Israel Dental Association. 2019;36:28-36.

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