COMPARING ENAMEL SURFACE MICROHARDNESS OF HUMAN TEETH WITH THREE OTHER ANIMAL SPECIES (HERBIVOROUS, CARNIVOROUS AND MARINES) ENCOUNTERING DIFFERENT ORGANIC ACIDS

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ABSTRACT

Aim: The composition of enamel may vary in different animal species. The mineral, organic and water content as well as the structure and architecture of enamel may affect the resistance of this tissue to organic cariogenic acid attack. The aim of this study was to compare the microhardness of human enamel with three other animal species (herbivorous, carnivorous and marine animals) in contact with three organic cariogenic acids.

Materials & Method: 15 sound teeth from each animal species as well as human were collected .The teeth were sectioned and ground in order to produce enamel samples 3*4 mm in dimension. The initial microhardness of samples were measured. In vitro demineralization lesions in three subgroups (a,b,c) were formed by immersion of the samples into a solution of 2% citric acid (pH= 2.6) , 99.7% acetic acid (pH= 3.1) and 73.5% lactic acid (pH=2.4) respectively for 30 minutes. After demineralization process microhardness of the samples were measured again and recorded. The collected data were analyzed by SPSS software.

Results: It was shown that there was statistically significant difference between initial enamel microhardness of four mentioned groups before demineralization process was statistically significant ($p<0.001$). after contact with three organic acids the amount of microhardness reduction of human and dog enamel was not statistically significant but the amount in sheep and Barracuda fish groups was. In general Barracuda fish group showed the least reduction in enamel microhardness in contact with two organic acids (lactic acid and acetic acid).

Conclusion: Based on the results of the present study, we concluded that the enamel of human teeth as well as herbivorous, carnivores and marine animals showed different resistance to demineralization process. In total, the teeth in Barracuda fish group (marine animal) showed the least reduction of enamel microhardness in contact with cariogenic acids.

Key words: Animal species, Enamel, Human, Microhardness, Organic acids.

Introduction

Dental enamel is a crystalline latticework composed of various minerals, the principal component of which is a complex calcium phosphate mineral called hydroxyapatite. Periodic exposure to organic acids made by carbohydrate fermentation with pH lower than 5.5 leads to dissolution of this mineral component and occurance of dental caries.[1]

Human dental enamel, which is the hardest tissue in the body, is composed of 92–96% inorganic matter, 1–2% organic material and 3–4% water by weight.[2] Most of the inorganic matter is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), the most abundant mineralized tissue in human and mammalian teeth).[3]

Enamel and dentin from different mammalian species have long been used as substitutes for human ones, since mammalian and human teeth are morphohistologically similar to each other.[4] The composition of enamel may vary in different animal species (herbivorous, carnivorous and marines).The mineral, organic and water content of enamel tissue in mentioned species may vary and hence their enamel resistance to organic cariogenic acids may be also different.

Some researchers have studied the chemical composition of human teeth enamel in comparison to other animals: bovine, porcine and ovine. The results showed that human teeth is the most mineralized. The most abundant chemical elements in enamel and dentin tissue of four mentioned species were calcium and phosphorus.[5]

Edmunds DH and his colleagues also evaluated human, bovine, equine, and ovine teeth enamel for studies on artificial bacterial carious lesions .The lesions were similar in appearance in all species when examined under polarized light, but there were structural differences between lesions in human and animal teeth.[6]

Although there are some researches that compare human dental hard tissue with other animals but still there are limited comprehensive data about dental enamel changes made by different organic acids in human, carnivores, herbivorous and marines.

The aim of this study is to compare the enamel surface microhardness of human, dog, sheep and Barracuda fish teeth according to Vickers hardness test and determine the effect of three cariogenic organic acids on enamel microhardness in these groups.

Materials & Method

The study Based on the pilot study 15 anterior teeth from each human and animal species were used (15 human, 15 dog, 12 sheep, 15 barracuda fish anterior teeth). All human teeth were collected after patients signed an informed consent, in accordance with the ethics committee of Shiraz University of Medical Science.

The animal teeth were collected from stem cell research center of Shiraz University of medical science and Barracuda fish were also available in stores. Intact teeth without any sign of caries were selected and stored in 0.2% tymol.

The teeth were sectioned transversally with a diamond saw (KgSorensen, Barueri, Brasil) and ground with a 600-grit silicon carbide paper under a stream of running water in order to produce enamel samples of 3*4 mm. The teeth were stored in deionized water during the sample preparation process. Specimens were mounted individually on 1-inch acrylic blocks and the initial microhardness of the samples were measured by Microvicker's hardness tester machine (Frank co,Germany).

In order to decrease the error rate the sample surfaces should be dry, smooth and without any curvature. The force and time of exertion in the machine was determined as similar researches and based on the specialist opinion. Then human and other animal enamel specimens were each divided into 3 subgroups (a,b,c) based on the type of demineralizing solution used. In vitro demineralization lesions in these subgroups (a,b,c) were formed by immersion into a solution of 2% citric acid (pH= 2.6), 99.7% acetic acid (pH= 3.1) and 73.5% lactic acid (pH=2.4) respectively for 30 minutes.

Specimens were rinsed with deionized water after demineralization and stored in 100% relative humid environment until further use. After demineralization process microhardness of the samples were measured and recorded.

The collected data were analyzed by adapting the SPSS package, version 18 (SPSS Inc., Chicago,IL, USA). To determine the effect of each acid on enamel microhardness reduction of 4 species (human, carnivores, herbivores and marine animals) One-Way ANOVA and Tukey HSD tests were used.

To compare the effect of three demineralizing solutions on enamel samples of each group Two-Way ANOVA and sub group analysis was done.

Result

Table 1 shows the mean initial enamel microhardness values of 4 groups. Because the variance of data is not homogenous the logarithm of data was taken and compared statistically.

Group	N	Mean Ln	St.Deviation	Min	Max
Human	15	5.7483	0.20323	5.25	6.05
Fish	15	3.9165	0.12934	3.67	4.17
Sheep	15	5.4505	0.21640	5.08	5.86
dog	15	5.5769	0.14853	5.34	5.86

Table 1: The mean Ln of baseline microhardness values

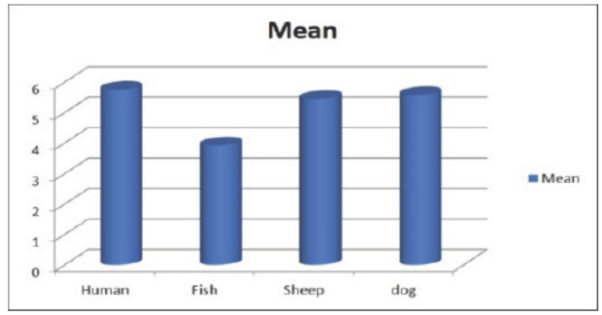

Figure 1: The mean Ln of baseline microhardness values

Based on One-Way ANOVA test results the difference of initial surface microhardness of 4 groups of study before demineralization process is statically significant ($p<0.001$). Pairwise comparison of data by Tukey HSD test showed that enamel microhardness of human group is higher than other groups and enamel microhardness of barracuda fish is the lowest. Dog and sheep enamel microhardness values are between these two groups and their difference is not statistically significant ($p=0.22$). (human>dog and sheep>fish).

The amount of reduction in enamel microhardness of all groups are shown in table 2.

acid \ group	1-Human	2-fish	3-sheep	4-dog
Lactic acid (subgroup a)	-40.02	-39.56	-187.40	-58.92
Citric acid (subgroup b)	-9.12	-23.84	-84.66	-83.64
Acetic acid (subgroup c)	-45.26	-24.56	-44.18	-71.84

Table 2: Microhardness changes after demineralization process.

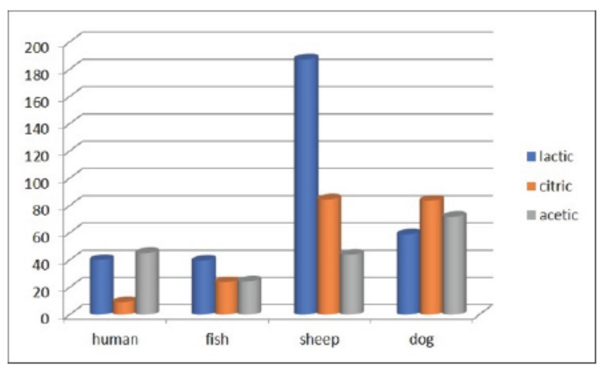

Figure 2: Microhardness changes after demineralization process.

Two-Way ANOVA analysis showed that the interaction effect between organic acids and dental enamel type is statistically significant ($p=0.041$), so subgroup analysis was done for comparing the amount of microhadness reduction after encountering different organic acids.

Comparing the effect of three organic acids on enamel microhardness reduction in each group of the study showed that just in groups 2 and 3 (Fish and Sheep) the difference is statistically significant (*p* values are respectively .006 and .015). In other two groups of the study (1 and 4) three organic acids act similarly and the difference is not meaningful (*p* values are respectively 0.712 and 0.824). In this regard in groups 2 and 3 the difference between subgroups a and b as well as a and c is meaningful. [Table 3 and 4]

Acid(I)	Acid(J)	Mean Difference	Std. Error	Sig
Lactic(a)	citric	-15.720	5.068	.023
	acetic	-15.000	5.068	.030
Citric(b)	lactic	15.720	5.068	.023
	acetic	.720	5.068	.989
Acetic(c)	lactic	15.000	5.068	.030
	citric	-.720	5.068	.989

Table 3: Effect of three organic cariogenic acids on Fish enamel

Acid(I)	Acid(J)	Mean Difference	Std.Error	Sig
Lactic(a)	citric	-102.740	36.531	.039
	acetic	-143.220	36.531	.005
Citric(b)	lactic	102.740	36.531	.039
	acetic	-40.480	36.531	.527
Acetic(c)	lactic	143.220	36.531	.005
	citric	40.480	36.531	.527

Table 4: Effect of three organic cariogenic acids on sheep enamel

Also comparing the effect of each organic acid on four types of enamel specimens(human ,fish ,sheep and dog) by One-Way ANOVA test showed that only the effect of lactic acid on the amount of microhardness reduction in human and each animal species is statistically significant (*p* value =0.002).But this effect is not seen for citric and acetic acid. (*p* values are 0.103 and 0.628 respectively)

Based on the results the amount of microhardness reduction of sheep enamel, encountering lactic acid, is the highest but fish enamel shows the least changes encountering this acid.

Discussion

There are few studies in the literature, which have investigated the differences between human and other animal teeth enamel susceptibility to demineralization or erosion. In the present study the effect of three cariogenic organic acids on enamel microhardness was not statistically significant for dog and human groups, but this effect was different for sheep and Barracuda fish groups.

Numerous studies have been carried out using bovine dental enamel as a substitute for human enamel when demineralization and remineralization protocols were investigated,[7-12] but data on other animal's teeth is still limited. In several other studies the enamel structure of various animal species have been evaluated and compared with human dental enamel.Based on the results of these studies enamel has different morphology and configuration in different animals that leads to variation of its radiodensity and microhardness. [7-13]

Based on the results of our study the difference of baseline microhardness between 4 groups(human and three other animals) before demineralization process is statistically significant . Microhardness of human enamel is higher than other groups and enamel microhardness of barracuda fish is the lowest. Dog and sheep enamel microhardness are between these two groups and the difference of them is not statistically significant.(human>dog and sheep>fish). Field *et al* also concluded that human, bovine and ovine enamel roughness and microhardness were significantly different with one another at baseline ($p < 0.001$).[14]

Analyzing the enamel chemical structure of different animals is helpful in caries research studies. In some researches the chemical composition of human tooth enamel has been studied in comparison to other animals: bovine, porcine and ovine. Their results showed that human tooth is the most mineralized considering the amount of calcium and phosphorus as the most abundant mineral elements in enamels and dentin in comparison to the three animal groups.[5]

Although there are limited studies on marine animal teeth enamel, some authors investigated the microhardness of shark teeth enamel. Vicker's microhardness tests showed that the hardness of shark teeth and human teeth was comparable considering both dentin and enamel/enameloid tissue. But data about other marine animals are limited.[15] In our study the enamel of Barracuda fish showed the least changes in microhardness after encountering lactic and acetic acid . This fact that marine's teeth consist of the harder biomaterial molecules fluoroapatite may be a reason for their resistance to demineralization process by organic acids. Fluoroapatite has different mechanical properties than hydroxyapatite: it has a higher bulk modulus than hydroxyapatite, higher stiffness constants and higher elastic modulus, and is totally harder than hydroxyapatite.

The configuration of enamel rods is also is different in enameloid tissue which is seen in marine animal teeth more than other groups.

Data about carnivorous animal teeth showed that the structure of inner and outer enamel layers differ between these species (dogs and cats) and human teeth .The growth lines of enamel in carnivores do not terminate at perikymata on the tooth surface.[16] The scarcity of dental caries in carnivores may be related to this structural specificity and contrasts with the high prevalence of periodontal diseases and calculus formation in them.[17]

Based on our results enamel microhardness of barracuda fish is not higher than human at base line but its magnitude of change in contact with lactic and acetic acid is the least. Identifying factors that participate in this matter would be worth mentioning in further studies about caries process. Higher intermolecular bond in fluoroapatite in their enamel tissue may be a reason for the less microhardness variations in contact with organic acids. The less susceptibility of enameloid tissue to acid dissolution may be another reason for microhardness variations in this groups.

Conclusions

Based on the results of the present study, we concluded that enamel specimens from human, marines, herbivores and carnivores showed significantly different characteristics in contact with cariogenic acids. The amount of microhardness reduction of sheep teeth enamel , encountering lactic acid was the highest and fish enamel showed the least changes encountering the same acid. The less susceptibility of enameloid tissue to acid dissolution as well as the presence of fluoroappatite molecules in their enamel tissue may be the reasons of less microhardness variations in fish group.

Suggestions

If we could provoke enameloid formation during amelogenesis period in human tooth buds,as well as fluoroapatite formation in human enamel crystals, just like marine teeth enamel ,we would have a much more resistant teeth against acid dissolution attacks and hence less caries occurance.

Aknowledgments

The authors thank the Vice-Chancellory of Shiraz University of Medical Science for supporting this research .This manuscript is based on the thesis number: 1478 by Dr Parisa Derakhshan and Zahra Mohammadi with advisory of Dr.Ali Nozari.

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