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IMPACT OF CARIOGENIC ACIDS ON OTOLITHS OF POMADASYS KAAKAN FISH IN THE PERSIAN GULF AND HUMAN TEETH ENAMEL : AN INVITRO STUDY

Ali Nozari[1*], Mohammad Javad Moazzamian[2], Maryam Pakniyat[3]

[1*]Assistant Professor, Department of Pediatric Dentistry, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Student research committee, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.
[3]Postgraduate Student, Department of Pediatric Dentistry, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.

[*]**Corresponding Author:** Ali Nozari
[*]Department of Pediatric Dentistry, Shiraz Dental School, Ghasrodasht Street, Shiraz, 7144833586, Iran, Fax:+987136270325, Phone Number: +987136263192, Email: nozariali133@gmail.com; nozariali133@yahoo.com

Abstract

Objective: This study aimed to assess the surface microhardness of otoliths taken from Pomadasys kaakan fish in Persian Gulf and analyze the destructive effect of some cariogenic acids (citric acid, lactic acid and pyruvic acid) on its surface hardness in different time intervals. The effect was compared with permanent human teeth enamel.

Method: This in vitro experimental study was conducted on 20 sound first premolar teeth which were extracted due to orthodontic reasons and 20 sound otoliths from Pomadasys kaakan fish. Otoliths were removed from the head of Pomadasys kaakan and cut to 30 pieces of 2×2×2 mm. Same cuts were taken from sound human permanent teeth. These cuts were stored in 100% humidity (normal saline solution) and at temperature between 25 to 30 degrees centigrade until the experiment. The specimens were divided into two groups (group 1= teeth, group 2= otoliths) and each group had three subgroups of 10 specimens which were immersed in citric acid, lactic acid and pyruvic acid accordingly. The surface microhardness of teeth enamel and otoliths were measured by Vickers microhardness tester at baseline (0), and at 5 and 30 min time intervals after immersion in the freshly prepared acidic solutions.

Result: Repeated measures ANOVA showed that the effect of immersion time on microhardness was significant ($p<0.001$). Comparison among 0, 5 and 30 minutes time points using Bonferroni adjustment for both groups also showed significant differences in microhardness at different time intervals for three types of acids ($p<0.001$). Evaluation of the effect of the type of acid on microhardness for both groups revealed that the microhardness changes was not significantly different in three groups of acids, although there was some difference in change of microhardness in case of pyruvic acid. Otoliths showed somewhat higher resistance in surface hardness under the effect of pyruvic acid compared to human enamel, but it was not significant. In case of lactic acid, the teeth showed somewhat higher resistance compared to otoliths but the difference was also not significant. ($p<0.001$).

Conclusion: Contact time for three types of acids from 0 to 30 min was significantly effective in reducing surface microhardness of both otoliths and human teeth enamel. Citric acid, lactic acid and pyruvic acid were all capable of demineralization and reduction of surface microhardness in both groups almost equally. In total, both groups of otholits and human enamel showed the same rate of demineralization and reduction in surface microhardness under the effect of cariogenic acids.

Keyword: Hydroxyapatite**,** Calcium carbonate, microhardness

Introduction:

Dental caries is the most prevalent chronic disease affecting human, irrespective of age, sex, race and socioeconomic status (1). It is initiated by the process of fermentation, in which the production of strong organic acids such as lactate, formate and pyruvate cause demineralization of susceptible dental hard tissue (2, 3). Dental caries affect both the majority of adults and children, from 60% to 90%. Repairing and replacing decayed teeth is extremely costly in terms of time and money, so this accounts for a major drain on the resources of healthcare systems in the world (4). Streptococus mutans, the main initiative cause of dental caries, strongly releases acids by fermentation of carbohydrates, leading to demineralization of the teeth (2). The earliest detectable stage of caries is the appearance of a white spot on the tooth. This is the first manifestation of cariogenic acid attacks on teeth enamel and reduce the surface microhardness significantly. Studies in this criteria are valuable as the process of caries is still reversible in this stage by remineralizing enamel tissue leading to an increase in surface microhardness again (5).

Enamel is the hardest and most mineralized tissue biologically made in human and animal body. It is composed of 96% mineralized substance and 4% water. The calcium phosphate in the form of hydroxyapatite makes the structure of mineralized part of enamel which is almost a large complicated formula (6).

In the process of caries, destruction of hydroxyapatite takes place as a result of some organic acid attacks and in overt destroyed lesions, substitution of this substance with new molecules is not possible, hence operative techniques and substances are used to reconstruct the lost tooth structure.

Crystalized calcium carbonate ($CaCO_3$), especially in the form of aragonite which is found in otoliths of some fishes is another hard, mineralized biologic substance with high calcium percentage, totally different from hydroxyapatite in structure. Other form of $CaCO_3$ are Calcite with trigonal geometry, and vaterite with hexagonal geometry of crystals. Gauldie et al reported that the hexagonal Vatrite or trigonal calcite may be substituted by orthorhombic aragonite, with more resistant structure under the influence of genetic factors. The orthorhombic aragonite form of $CaCO_3$ found in otoliths is the most stable and resistant variety of this formula comparable with hydroxyapatite with a simpler construction (7-8). Sollner et al found that Stormaker and Otolin-1 genomes may be considered as the main reason for crystal changes of otoliths in Zebra fish (9).

Pomadasys kaakan fish has usually a sea coast ecology and lives in depth of up to 75 meters. Geographical ecology of this fish is reported in the West coast of Africa, Red Sea, Gulf of Aden, Sri Lanka, Northern Taiwan and all regions of Persian Gulf and Sea of Oman. It's trade name is Javelin grunter and has a rectangular body and has two holes under the chin with middle cavity. The fish teeth are small and tipped and the outer rows of teeth have been elongated (10-11).Otoliths or ear stones are composed of calcium carbonate, inorganic impurities and a protein matrix, and are located in the labyrinth of the ear in Osteichthyes of fish. These are considered the hardest biologic materials as well as the teeth enamel in the fish body (12).

There are three types of otoliths that help fish in balance and hearing: Sagitta, Asteriscus and Lapillus. Sagitta is the largest otolith in pomadasys kaakan. The composition of the three types are the same but the structural figure different (13).

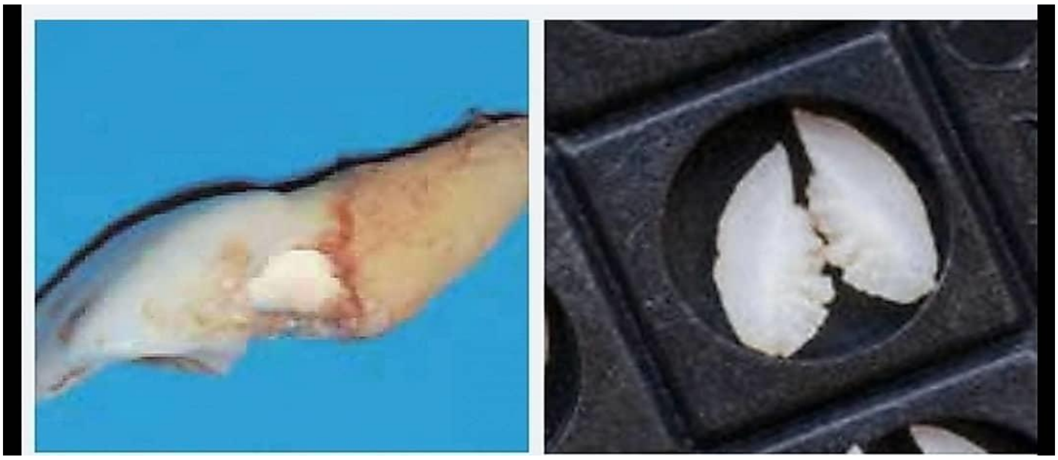
Description of sagitta otoliths in pomadasys kaakan: Its Shape is oval, with convex medial face and slightly concave lateral face due to moderate thickness of its central portion. The dorsal margin is angular and ventral margin round. Its excisura is moderately wide, pointing anteriorly, without

notch. Smaller samples of otholith <8 mm have a shallow, very acute and pointing upwards excisural notch on the dorsal margin near anterior ostium (14, 15).
Comparison of surface hardness of this biologic calcified tissue with the hardest and most resistant tissue of the body (enamel) and definition and comparing its structural formula with enamel may lead us to some ways increase the resistance of enamel structure to cariogenic acid attacks or even substitution of hydroxyapatite structure in destroyed enamel tissue and reconstruction of this important tooth formula in further researches.

Method and materials
This in vitro experimental study was conducted on 20 sound first permolar teeth which were extracted due to orthodontic reason and 20 sound sagitta otoliths of pomadasys kaakan. Otoliths from head of pomadasys kaakan were removed and cut to 30 pieces of 2×2×2 mm. Same cuts were taken from sound human permanent teeth. These cuts were made with diamond bur and were polished with fine abrasive paper and stored in 100% humidity (normal saline solution) at temperature between 25 to 30 degrees centigrade until the experiment. We divided our specimens in to 2 groups (group 1= teeth, group 2= otoliths) and each group were divided into 3 subgroups each containing 10 specimens. The specimens of both groups immersed in citric acid, lactic acid and pyruvic acid with PH values adjusted at 3 (Table1). The surface hardness of the enamel and otoliths were measured by Vickers microhardness tester (Sctmc, mhv-1000z china) under 100g load at time intervals of 0 at baseline, 5 and 30 min after immersion in the freshly prepared acidic solutions (T0, T5 and T30 respectively). We used Mann-whitney test and Wilcoxon signed rank test for statistical analysis.

Table 1. Characteristics of three acid solutions.

Acid	pH	Molarity	Degree of purity
Citric acid	3	0.047	2%
Lactic acid	3	9.9	85%
Pyruvic acid	3	1.2	33%



Tooth enamel **Otolith**

Result:
Overal 180 measurement on both groups of teeth and otoliths were done. Statistical analysis showed that in all groups increasing the time period of contact with acids, decreased the surface hardness significantly ($p \leq 0/05$). Repeated measures ANOVA showed that the effect of immersion time on microhardness was significant ($p<0.001$). At zero time and before contact with acids, both groups had almost the same surface hardness which means that otoliths had the same surface hardness as human permanent teeth enamel. Comparison of time intervals (0, 5 and 30 minutes) after immersion in acids using Bonferroni adjustment for both groups showed also significant differences in

microhardness at different time points for all three types of acids ($p<0.001$).This means that both groups showed a decrease in surface hardness after contact with acids. Comparison of changes in surface hardness of both groups, revealed that the microhardness was not significantly different under the effect of three acids, although from 5 to 30 minutes after immersion in Pyruvic acid, otolith showed somewhat higher resistance in surface hardness compared to teeth enamel (Figure1). In case of lactic acid, teeth enamel showed a slightly higher surface hardness resistance compared to otolith in the same time interval, but these changes were not statistically significant. ($p>0.001$)(figure2). Both the enamel and otolith showed almost the same variations in surface hardness in contact with citric acid (figure3).

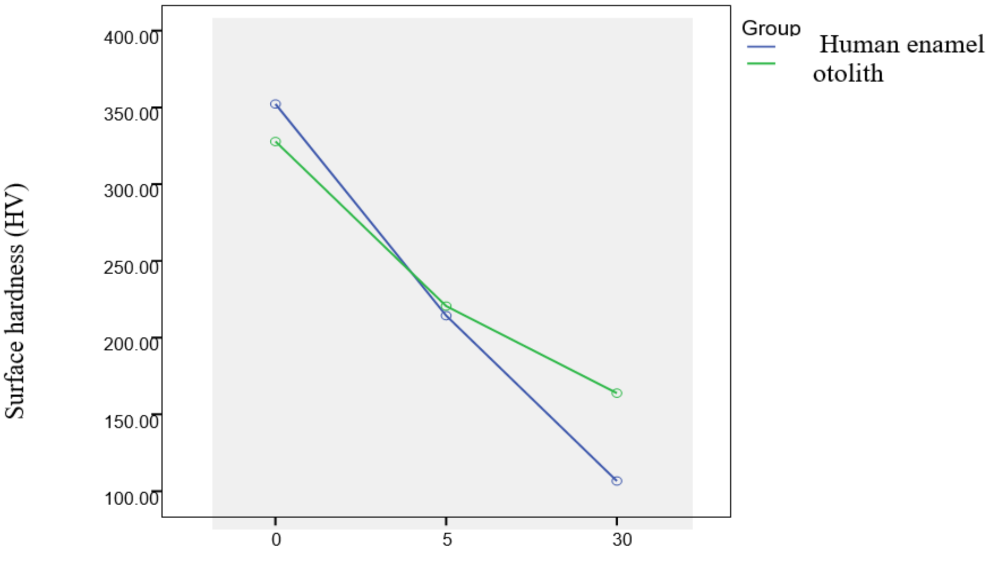


Figure 1: Decrement of surface hardness in permanent teeth enamel and otolith after contact with Pyruvic acid solution in different time intervals

Otolith shows somewhat more resistance to microhardness changes specially after 5 minutes of acid immersion

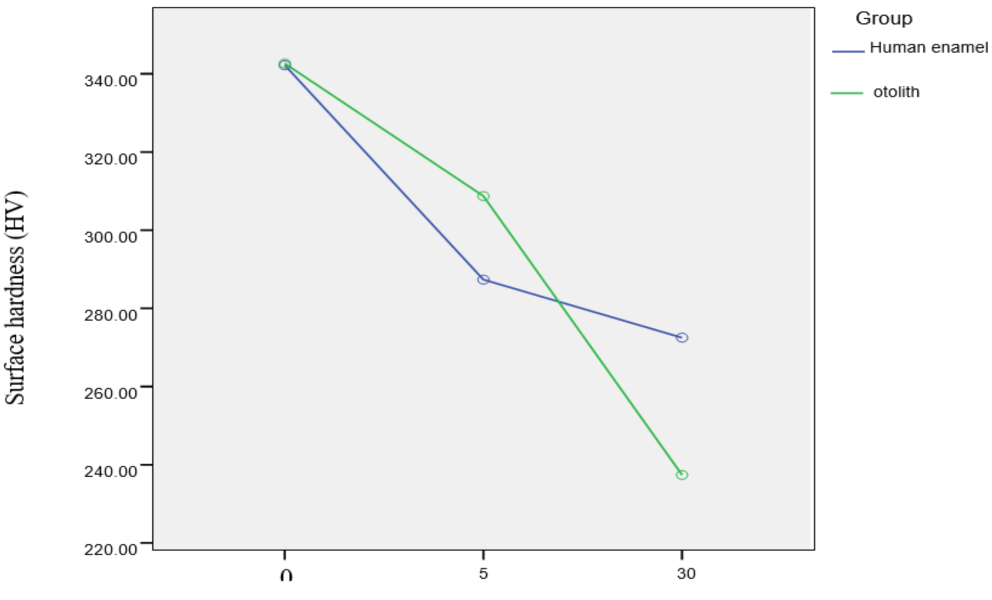


Figure 2: Decrement of surface hardness in permanent teeth enamel and otolith after contact with Lactic acid solution in different time intervals

Enamel shows somewhat more resistance to microhardness changes specially after 5 minutes of acid immersion

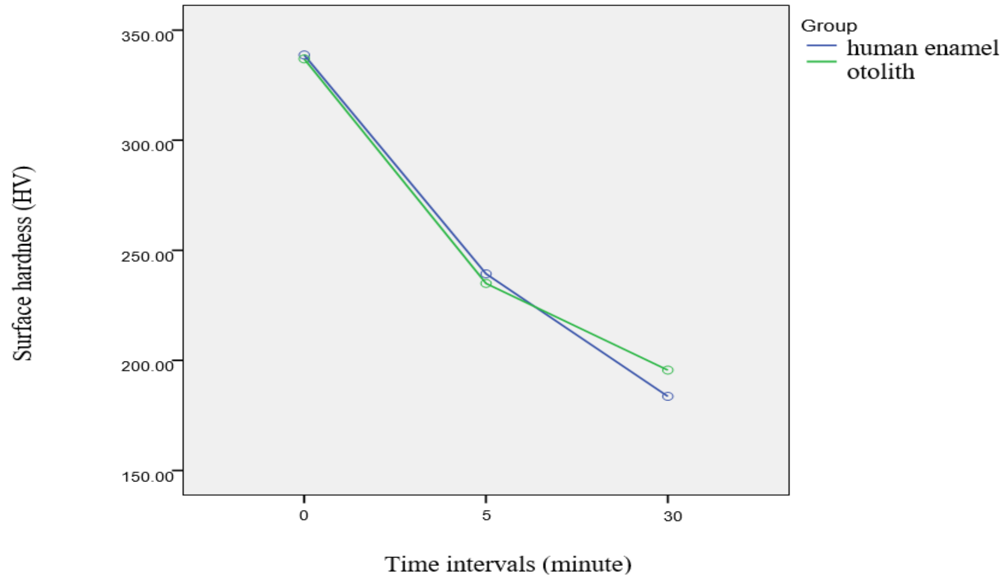


Figure 3: Decrement of surface hardness in permanent teeth enamel and otolith after contact with Citric acid solution in different time intervals

Enamel and otolith showed almost the same resistance to microhardness changes in both time intervals after acid immersion

Discussion:

Demineralization and remineralization of tooth is a continuous process. When demineralization exceeds remineralization process, teeth cavitation and caries will appear. Demineralization usually starts under the effect of organic acids that are the product of bacterial fermentation of food particles and leads to dissolution and softening of hard tissue of the teeth, the depth of which in enamel of permanent teeth may vary from 0.2 to 3 μm (16).

Otoliths are composed mainly from crystals of calcium carbonate with aragonite structure in orthorhombic figure which is the most rigid, resistant form of this chemical formula comparable with hydroxyapatite crystals in enamel (17). In this study, otolith crystals showed somewhat more resistance to dissolution in contact with pyruvic acid after 30 minutes compared to enamel crystals. It seems that separation of CO3 ion from calcium in aragonite molecule structure is somewhat more difficult by pyruvic acid with three H^+ ions compared with PO4 ion from calcium in hydroxyapatite molecules, though it was not significant.

Inversely, lactic acid with five H^+ ions could separate CO3 ion in otoliths somewhat easier than po4 ion from calcium in enamel accordingly, though not significantly.

Resistance to acid dissolution and maintenance of the surface hardness of teeth enamel and otoliths can be due to different reasons such as structural composition or presence of different elements in their chemical formula (18). In this study, both caco3 and hydroxyapatite crystals showed the same surface hardness before acid contamination in otolith and enamel accordingly.

With increasing the time of contact from 0 to 5 and to 30 minutes, the dissolving effect of all three acids elevated and hence, the surface hardness of both enamel and otholith crystals were decreased. Three examined acids (citric, pyruvic, lactic) acted almost the same as the effect of H^+ ion attacking to calcified substance increased with time.

Otoliths have some metallic elements like Sr, Ba, Se, Mg, Mn and, Al. According to double day studies in 2014, Sr is one of the most important elements in otoliths that can substitute calcium ion in caco3 crystals (19). Thomas et al found that Sr is more eager to substitute ca ions in aragonite structures which is present in otoliths and Mn ion in Vaterite structures (20). Sr is considered an important element, effective in surface hardness of crystals especially when combined with CO3 ion (21). Sr can substitute calcium ion in otolith crystals but Mn and Zn intrude crystals by absorption between caco3 molecules (19). According to a study by Antao et al by high-resolution power X-ray (diffraction), $CaCO_3$ has a more irregular and less resistant structure in comparison with $SrCO_3$, $PbCO_3$ and $BaCO_3$, so the mentioned ions can substitute calcium ions more easily and combine with CO_3 ions to make a more resistant molecule (21).

Ghadimi et al found in their research that some elements like Mn, Ti and Pb are effective in enamel crystal size and Ni, Cr, Se in structure form of the crystals. The physical characteristic and hardness of the crystals are hence changed after insertion of these ions specially Ti (22).

Kis et al in 2021 found that minor component magnesium incorporation into the prismless layer of sound primary enamel can affect hardness properties of human primary dental enamel. The increased Mg concentration in the dental enamel surface was followed by a notable increase of nanohardness (23).

Mohammed et al in 2015 concluded that Zn ion in the oral fluids can protect against enamel demineralization during an acidic challenge (24).

Krishnan et al in 2015 concluded that strontium (Sr) doped nano hydroxyapatite as a surface application modality enhances dental enamel remineralization/ repair in incipient carious lesions (25).

Wang et al in 2019 found that Addition of the Sr ions to the acidic solution can decrease the dissolution of the enamel and prevent the enamel surface hardness loss in acidic environment (26).

Reitzneroua et al found that the concentration of metallic ions are overally more in the first 50µ of enamel surface which is effective in microhardness of this mineralized tissue (27). So, if we can insert some metallic ions, especially Sr. which is present in aragonite structure of otoliths into hydroxyapatite crystals of surface enamel, the microhardness of this dental tissue and hence the resistance of this biologic hard tissue to acid dissolution will be increased.

Conclusion:

Citric acid, lactic acid and pyruvic acid were all capable of demineralization and reduction of surface hardness in both enamel and otoliths, almost equally after 5 and 30 minutes. Before contact with acids, otoliths had almost the same surface hardness as human permanent teeth enamel. Under the effect of these acids there were no significant difference between two groups from the point of acid solubility and both groups showed the same rate of demineralization and reduction in surface hardness. The results of this study can be used for further in vitro investigations.

The metallic inclusion of both otolith and enamel tissues maybe considered as an important factor for increasing surface hardness and resistance of these organs against chemical attacks. Further researchers are needed in this area.

Suggestion:

As the construction of caco3 molecules is more simple and much more practical to make in laboratory than hydroxyapatite crystals, it is worth to find a way for substitution of lost enamel structures by these molecules in the form aragonite in further researches. Aragonite calcium carbonate may also be considered to be used in operative dental materials for filling and reconstruction of destroyed enamel tissue due to caries in further researches. Inserting some metallic ions like strontium (Sr) in the enamel crystals such as seen in otoliths may also be effective in increasing the surface hardness of this tissue and hence its resistance to caries.

Conflict of interest: The authors declares that there is no conflict of interest

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