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Effect of Zamzam Water on the Microhardness of Initial Carious Lesion of Permanent Teeth Enamel (An in vitro study)

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Abstract

Chemical tests concluded that Zamzam water is absolutely good and suitable for drinking as it has very high percentage of sodium, calcium, magnesium, and many other minerals. The purpose of this study was to test the effect of Zamzam water on the microhardness of permanent teeth enamel.

Teeth samples (n= 30) with artificially initiated carious lesions were divided randomly into three groups, study group treated with Zamzam water (n= 10), control positive treated with sodium fluoride (n=10), and control negative treated with deionized water (n=10). Teeth were subjected for microhardness assessment before and after pH cycling and treatment with the three agents.

The microhardness of enamel surfaces treated with Zamzam water is shown statistically significant increase in the values following demineralization which was not different from that of sodium fluoride.

Zamzam water cause an increase in the microhardness of the enamel surface after pH-cycling.

Key words: Zamzam water, Microhardness, pH- cycling.

Introduction

Dental caries is one of the most prevalent oral health problems especially among children and young adults [1, 2]. Primary prevention of dental caries is directed toward restriction of cariogenic diet, dental plaque control through individual and professional oral hygiene measures as well as the increase of resistance of teeth to acid attack [3]. Increasing the tooth resistance to acid attack can be achieved by fluoride that gives hardness and durability to the tooth enamel and protect against caries [4, 5]. Elements as Zinc, Copper, and Strontium were reported to have cariostatic effect by some studies, the same elements were seen to have no or inverse association to dental caries by others [6, 7, 8]. Water is one of the main dietary components, its quality plays an important role for the health of human being, the use of Zamzam water is widely practiced [9]. According to Arab historians, the Zamzam Well has been in use for around 4000 years. Zamzam well is located in the holiest mosque of the Muslims in the city of Makkah, which is in the western province of the Kingdom of Saudi Arabia. The well is four thousand years old and the story of its formation is well known to Muslims. It is approximately 40 meters deep and surrounded by hills of igneous rocks. Drinking water from Zamzam well has special significance for Muslim peoples. Millions of Muslims drink this water as sacred water, especially during pilgrimage and Umrah each year [10]. In 1971, the Ministry of Agriculture and Water Resources sent samples of Zamzam water for investigations to the European laboratories to test the potability of the water, the results of the water samples tested by the European laboratories showed that Zamzam water has a special physique that makes it advantageous water [11]. The main difference between Zamzam water and other water (city water) was in the quantity of calcium, sodium, potassium and magnesium salts, the content of these was slightly higher in Zamzam water, but more significantly, the water contains fluorides that have an effective germicidal action [12]. Hydrochemical studies of Zamzam water have indicated that it is a sodium chloride water and of meteoritic origin. The four toxic elements arsenic (As), cadmium (Cd), lead (Pb), and selenium (Se) have been found below the danger level for human consumption. Chemically Zamzam water is suitable for drinking purposes [13].

This study was designed in order to test the effect of Zamzam water on the microhardness of the artificially initiated carious lesion of outer enamel surface in comparison to deionized water, and sodium fluoride agent (0.05% solution).

Materials and Methods

Teeth sample consisted of 30 maxillary first premolars, extracted from 12-14 years old patients, referred from Orthodontic Department, College of Dentistry University of Baghdad.

Teeth were washed with deionized water to remove any debris and stored in 20 ml deionized water to which 0.1% thymol added, then kept in refrigerator at 4 °C until use [14].Teeth samples were divided randomly into study group (n= 10), positive control group (n=10), and negative control group (n=10). All teeth were subjected for microhardness assessment before any processing. The initial microhardness was determined by Vickers Microhardness Device at a load of 500 gram.

Buccal and lingual surfaces of each tooth were coated with an acid resistant nail varnish leaving a circular window approximately six mm in diameter. The position of each window on tooth surface was standardized using orthodontic ruler. Teeth were adapted in an acrylic model, and the root of each tooth was covered with a red wax till the cervical line. The window of each tooth surface was ground and polished using a wet emery grit-paper. The grit paper was placed in special manual device. This procedure allowed a flat surface of each tooth for microhardness testing [15].

The pH–cycling procedure was followed for the induction of caries lesion on enamel surface [16]. Demineralization and remineralized solutions were prepared and pH of demineralized solution was adjusted to 4.3 at 37 °C and remineralized solution was adjusted to 7 at 37 °C.

Each tooth was immersed in 20 ml of the demineralization solution and kept for 6 hours at 37 °C in the incubator .Teeth were withdrawn and rinsed with running deionized water for one minute, there after each tooth was immersed in a remineralizing solution for 17 hours and kept in an incubator at 37 °C .This procedure was repeated daily for ten days [17]. After pH cycling, the initial microhardness was reassessing for all teeth. Then each group of teeth (study group and control groups) was treated as follow:

For study group each tooth was immersed in a 30 ml of Zamzam water for 4 minutes, then rinsed with deionized water for two minutes and restored in deionized water for the next day at 37°C in the incubator, this procedure was repeated daily for one week, and samples were re-examined for microhardness by Vickers Microhardness Method.

For control negative group teeth were treated as in study group except that deionized water was used instead of Zamzam water. Microhardness was determined as in study group. Concerning control positive group teeth were treated with 0.05% sodium fluoride. Microhardness also determined as above.

Statistical analysis:

1. Calculation of the statistical parameters, mean and standard deviation.
2. Student's – test, and analysis of variance (ANOVA) for calculating the significance of differences between the different variables. The Confidence limit was accepted at 95%.

Results

The microhardness mean values of the sound enamel surfaces, then after pH cycling procedure and after treatment with 0.05% sodium fluoride are seen in Table(1). The difference between the microhardness for the three variables was examined by ANOVA test which is shown in Table (2), a highly significant difference was shown. LSD test was performed to compare each two variables; there was a statistical highly significant reduction in microhardness of enamel following the pH cycling procedure, while a significant increase in the microhardness was illustrated after treatment with 0.05% sodium fluoride (Table 3).

Table (4) shows the mean values of the microhardness of sound enamel surfaces, after demineralization and following treatment with Zamzam water. A highly significant difference between the three variables of microhardness was seen by ANOVA test Table (5). LSD test revealed a highly significant difference between the initial microhardness values of the sound enamel surfaces and that after the pH–cycling procedure (demineralization),while a significant increase in the microhardness following the treatment with Zamzam water was seen (Table 6).

The mean values of microhardness of enamel before and after pH cycling and following treatment with deionized water are shown in Table (7). ANOVA test revealed a highly significant difference between the three variables Table (8), although there was a highly significant decrease in the microhardness after demineralization, the microhadness showed only a slight increase after treatment with deionized water which was not statistically significant from the values after deminealization (Table 9).

Table (10) shows a comparison between the mean values of microhardness of study and control groups using student's t- test, a non significant difference in the microhardness was seen when Zamzam water compared to sodium fluoride, while a highly significant difference in the microhardness between Zamzam water and deionized water was revealed.

Discussion

The primary prevention of dental caries involves the increase in the resistance of the outer enamel surfaces to acid dissolution and enhancement of remineralization. Fluoride has been widely used since the thirties of last century for the prevention of dental caries [4, 3, 8], however, the effect of other elements in relation to dental caries is not well substantiated. It is well documented from the experimental studies that sodium fluoride is effective in the remineralization of initial carious lesion and increase the resistance against caries attack [18, 19, 20]. For this reason sodium fluoride was chosen in the present experiment as a positive control while deionized water was used as a negative control .The pH cycling methods of Featherstone et al [16] was followed for the initiation of carious lesion. This method is simple reliable and successful, it can provide standardization for the worker in these types of experiments.

In the present study the microhardness of the sound enamel surface was measured for the buccal surface due to a higher value of microhardness that was recorded in the buccal surface compared to other surface. This may be attributed to variations in the mineral compositions of the surfaces [21]. Another explanation was mentioned by Fabiano et al [22], who attributed the difference to the variation in the orientation of crystals between buccal and lingual surfaces, for this reason buccal surface was chosen for this experiment.

The microhardness of the enamel surface following the pH–cycling method showed a statistically highly significant reduction in the value of the microhardness for the study group and positive control group (Zamzam water and sodium fluoride) respectively. This was an indication of the demineralization of enamel surface.

Following application of 0.05% sodium fluoride, an increase in the microhardness was detected, statistically this increase was significant. This may indicate a remineralization of initial carious lesion, it is assumed that fluoride ions reacted with the hydroxyl appetite crystals and a new crystalline product was formed which is differ from the fluoroapetite [3, 23]. This might cause the increase in the microhardness values seen in this study. Fluoride was reported to react not only with hydroxyapetite crystals in the surface layer but also in the subsurface layers and ultimately has a greater crushing strength than the original demineralised material [24, 25].

In the present study, Zamzam water was also successful in the increase of the micohardness value of demineralised surface. Incorporation of Zamzam water elements (fluoride, magnesium, calcium) in the appetite crystals may in one way or another favour increase the resistance to acid dissolution. The end result of chemical reaction is not well understood, however presence of fluoride components in Zamzam water may be responsible for the chemical reaction between Zamzam water constituents and appetite crystals. For all the study and control groups the microhardness values following treatment with different solutions were always less than the values of sound enamel surface. This may give an indication that none of the solution tested was able to increase the microhardness until reaching the original values before pH- cycling. This may be attributed to the (time) of application followed in the present experiment .Teeth were treated for four minutes for seven days period, increasing the time of treatment to weeks rather than one week may increase the microhardness values , however this may need to be confirmed by further studies.

Changes in the microhardness of the demineralised surface following the treatment with Zamzam water were compared with the control negative and that of control positive, although sodium fluoride 0.05% gave the highest value than Zamzam water, the difference was not significant, the least change was recorded for deionized water, one can expected from all these tests that Zamzam water is effective in remineralization of initial carious lesion and its effectiveness is not different from that of sodium fluoride. However, the cariostatic potential of Zamzam water need to be confirmed by further studies (experimental as well as human studies on an artificial as well as natural carious lesion) before giving any recommendation of using Zamzam water in the dental practice.

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Table (1): Microhardness (Mean Values and Standard Deviation) of Enamel Surfaces Treated by Sodium Fluoride.

Microhardness of Enamel	Mean	S D
Normal Enamel Surface	285.40	66.933
Demineralized Enamel	30.910	7.443
Remineralized Enamel	69.018	6.911

Table (2): ANOVA Test among Variables of Microhardness for Sodium Fluoride.

	Sum of square	d.f.	Mean square	F- value	P value
Between groups	376678.2	2	188339.075	123.286	0.000
Within groups	41246.898	27	1527.663		
Total	417925.0	29			

** Highly Significant P<0.001.

Table (3): Least Significant Difference (LSD) Test between Each Two Groups for Microhardness of Sodium Fluoride.

Group 1	Normal enamel surface		Normal enamel surface		Demineralized enamel	
Group 2	Demineralized enamel		Remineralized enamel		Remineralized enamel	
	Mean difference	P	Mean difference	P	Mean difference	P
	254.4300*	0.000	216.3820*	0.000	38.0480*	0.038

Significant P<0.05, Highly Significant P<0.001

Table (4): Microhardness (Mean and Standard Deviation) of Enamel Surfaces Treated by Zamzam Water.

Microhardness of Enamel	Mean	SD
Normal Enamel Surface	301.80	52.715
Demineralized Enamel	34.390	7.032
Remineralized Enamel	60.650	4.926

Table (5): ANOVA Test among Variables of Microhardness for Zamzam Water.

	Sum of square	d.f.	Mean square	F- value	P value
Between groups	432250.1	2	216125.030	217.437	0.000
Within groups	26837.074	27	993.966		
Total	459087.1	29			

Table (6): Least Significant Difference (LSD) Test between Each Two Groups for Microhardness of Zamzam Water.

Group 1	Normal enamel surface		Normal enamel surface		Demineralized enamel	
Group 2	Demineralized enamel		Remineralized enamel		Remineralized enamel	
	Mean difference	P	Mean difference	P	Mean difference	P
	268.4100*	0.000	238.1500*	0.000	30.2600*	0.041

Table (7): Microhardness (Mean Values and Standard Deviation) of Enamel Surfaces Treated by De-Ionized Water.

Microhardness of Enamel	Mean	S D
Normal Enamel Surface	298.80	56.887
Demineralized Enamel	50.070	5.888
Remineralized Enamel	53.155	2.074

Table (8): ANOVA Test among Variables of Microhardness for Deionized Water.

	Sum of square	d.f.	Mean square	F- value	P value
Between groups	407392.0	2	203695.994	186.583	0.000
Within groups	29476.303	27	1091.715		
Total	436868.3	29			

Table (9): Least Significant Difference (LSD) Test between Each Two Groups for Microhardness of Deionized Water.

Group 1	**Normal enamel surface**		**Normal enamel surface**		**Demineralized enamel**	
Group 2	**Demineralized enamel**		**Remineralized enamel**		**Remineralized enamel**	
	Mean difference	**P**	**Mean difference**	**P**	**Mean difference**	**P**
	248.7300*	0.000	245.6450*	0.000	3.0850	0.836

Table (10): Comparison of the Microhardness Mean Values between the Control and Study Groups.

Groups	t-value	p-value	Description
Zamzam water× Sodium fluoride	1.325	0.202	NS
Zamzam water× Deionized water	4.501	0.000	HS

d.f. = 18