Effect of Zamzam water on the microhardness of initial caries-like lesion of permanent teeth, compared to Casein Phosphopeptide-Amorphous Calcium Phosphate agents

Athraa’ M. Al-Weheb B.D.S, M.Sc. (1)
Ali Hadi Fahad B.D.S, M.Sc. (2)

ABSTRACT
Background: chemically Zamzam water is suitable for drinking purposes contains calcium, magnesium, sodium, fluoride and other salts higher than other water that have an effective germicidal action. The aim of this study was to investigate the effect of Zamzam water on the microhardness of initial carious lesion compared to CPP-ACP agents.

Materials and methods: thirty two maxillary first premolars with enamel caries-like lesion randomly divided into one study group treated with Zamzam water and three control groups CPP-ACP, and CPP-ACP+NaF as a positive control and deionized water as a negative control (each group consists of 8 teeth). Teeth were subjected for microhardness assessment before and after pH cycling and treatment with the selected agents.

Results: Agents of study groups were statistically highly significant in elevation of the microhardness values, CPP-ACP+NaF caused highest change in the microhardness (158.58%) and less for CPP-ACP (81.48%) and lesser for Zamzam water (80.97%).

Conclusions: Zamzam water was effective in remineralization of the outer enamel caries-like lesions, which was reflected by increase in enamel microhardness values.

Key words: Zamzam water, pH-cycling, CPP-ACP, remineralization.

INTRODUCTION
Water is one of the main dietary components. Its quality plays an important role for the safety of food particularly for infants (1). Millions of Muslims drink Zamzam water as sacred water, especially during pilgrimage and Umrah each year. 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 4000 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 (2). The results of the water samples tested by the European laboratories showed that Zamzam water has a special physique that makes it advantageous water, that the main difference between Zamzam water and other water (city water) was in the quantity of calcium and magnesium salts, the content of these was slightly higher in Zamzam water. Additionally, the water contains fluorides that have an effective germicidal action. Moreover, the remarks of the European laboratories showed that the water was fit for drinking (3). The preponderance of evidence indicates that fluoride can reduce the incidence of dental caries and that fluoridation of drinking water can provide such protection (4,5).

Multi-elemental and hydrochemical compositions of the holy Zamzam water have been studied. A total of 34 elements have been found with calcium (Ca), magnesium (Mg), sodium (Na) and chloride (Cl) in the highest concentrations (6). The traditional understanding of the impact of diet on dental caries has focused on the importance of fermentable substrates in caries causation; however in the past 15 years there has been an increasing awareness of dietary components which can have hypo- or anticariogenic effects. These agents can be classified as either "active" or "passive" in terms of their caries preventive effects (7).

It is now clear that milk and milk products contain a variety of agents which can suppress caries progression and some which can exert "active" caries preventive effects. In the former group are protein buffers, calcium and phosphate ions, and whey proteins. Phosphopeptides have attracted considerable attention as caries preventive agents, and there is now a large body of evidence which indicates that phosphopeptides can modulate the mineralization of hydroxyapatite (8,9). In recent years, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been demonstrated to have anticariogenic properties in both laboratory animal and human in situ experiments. Casein phosphopeptides (CPP) are peptides derived from the milk protein casein that are complexed with calcium and phosphate (10,11). The CPP-ACP and fluoride have been
shown additive effects in reducing caries. This study was designed in order to test the effect of Zamzam water on the microhardness of the artificially initiated carious lesion of the outer enamel surface in comparison to casein phosphopeptide-amorphous calcium phosphate agents.

MATERIALS AND METHODS

Teeth samples in this study consisted of 32 maxillary first premolars extracted from 11-14 years old patients, referred for Orthodontic Department, College of Dentistry, University of Baghdad. Teeth were washed with de-ionized water, and then each tooth was wiped with acetone to remove any debris, then stored in 20 ml of de-ionized water to which 0.1% thymol was added to prevent microbial growth. Then teeth samples were kept in refrigerator at 4°C until use.

Teeth were divided randomly into one study group and three control groups and each group consisted of eight teeth. Enamel microhardness was measured initially for normal enamel and after induction of caries lesion by pH cycling procedure, and finally after treated by the selected agents (Zamzam water, CPP-ACP, and CPP-ACP+NaF). The microhardness measurement was done by Vickers microhardness device in the Department of Mechanical Engineering, University of Baghdad at a load of 500 gm for 30 seconds. A position of circular window of 6mm in diameter on the buccal surface of each tooth was standardized using orthodontic ruler, then an adhesive tape circle of 6mm diameter was cut and burnished on the buccal surface of the tooth using burnisher, after that an acid resistant nail varnish was used to paint the surfaces of the tooth, the adhesive tape was removed leaving a window on the buccal surface. Teeth were adapted in an acrylic model (the size of this model was 30 × 27 mm) using a red wax. The grit paper (grit 400) was placed in special manual device. Window of each tooth was ground and polished ten times in one direction. This procedure allowed a flat surface of each tooth for microhardness testing.

The Induction of Caries like Lesion on the enamel surface was conducted by preparation of demineralizing and remineralizing solutions and adjustment of pH. The demineralizing solution, which contained 0.075 M/L acetic acid, 1 mM/L calcium chloride, and 2 mM/L potassium phosphate had the pH adjusted to 4.3, while the remineralizing solution, which contained 150 mM/L potassium chloride, 1.5 mM/L calcium nitrate, and 0.9 mM/L potassium phosphate had a pH of 7. The pH cycling procedure was involved 6 hours of demineralization with 17 hours of remineralization, the procedure was repeated for a period of ten days, one time each day.

Statistical Analysis: Descriptive statistic including means and standard deviation. Estimation of the significance of differences among mean values using ANOVA and LSD tests. Confidence limit was accepted at 95%.

RESULTS

The mean values of the microhardness of the sound enamel surfaces, after demineralization and following treatment with Zamzam water, CPP-ACP and CPP-ACP+NaF are seen in Table (1). Statistically highly significant differences were recorded between different states of enamel for three agents. By using LSD test among variables (three steps) of microhardness, there is a highly significant reductions in enamel microhardness were observed after demineralization for all agents. A noticed increase in the microhardness values was seen after treatment with these three agents. These increases were statistically highly significant, Table (2). The mean values of the microhardness of enamel before and after pH cycling procedure and following the treatment with de-ionized water are shown in Table (3). ANOVA statistical test showed a highly significant difference between the three variables, although there was a highly significant reduction in values of enamel surface microhardness after pH cycling, the microhardness showed only a slight increase after treatment with deionized water which was statistically not significant Table (4).

However, none of the mentioned agents able to increase the elevation of the microhardness values from sound enamel, which is statistically significant, Figure (1). Figure (2) shows the changes in the microhardness values after treatment with selected agents estimated by special equation. Values from this figure reflect a very minor change in the microhardness for de-ionized water in comparison to other agents. CPP-ACP+NaF caused the highest change (158.58%), and less for CPP-ACP (81.48%) and lesser for Zamzam water (80.97%).

DISCUSSION

The chemical analysis of Zamzam water demonstrated highly significant readings in all inorganic elements including higher levels of fluoride, calcium and magnesium. Exposure to
flouride in drinking water has been shown to be beneficial for oral and general health, especially in relation to dental caries (16). Ionic calcium in water is the best form to use to insure its proper absorption by the bones and teeth (17).

An interesting result recorded in this study was the higher microhardness values for CPP-ACP+NaF and less for CPP-ACP and lesser for Zamzam water. This can be explained by The CPP-ACP and fluoride has been shown to have additive effects in reducing caries experience. The additive anticariogenic effect of the 1.0% CPP-ACP and 500ppm fluoride in the rat caries experiments led to the investigation of the potential interaction between the CPP-ACP and fluoride. The fluoride ion had incorporated into the ACP phase that was stabilized by the CPP to produce a novel amorphous calcium fluoride phosphate phase (ACFP) at the tooth surface. The identification of this novel amorphous calcium fluoride phosphate (ACFP) phase led to the proposition that the formation of this phase is responsible for the observed additive anticariogenic effect of CPP-ACP and fluoride (18,19).

In this study, Zamzam water recorded increase in the microhardness value of demineralized surface and this may be due to incorporation of Zamzam water elements (fluoride, magnesium, and calcium) in the appetite crystals increasing the resistance to acid dissolution. However presence of fluoride components in Zamzam water may be responsible for the chemical reaction between Zamzam water constituents and appetite crystals (20). Changes in the microhardness of the demineralized surface following the treatment with Zamzam water were compared with CPP-ACP agents, although CPP-ACP+NaF gave the highest value than Zamzam water, but the difference between Zamzam water and CPP-ACP was not significant, the least changes was recorded for deionized water, one can expected from all these tests that Zamzam water is effective in remineralization of initial carious lesions and its effectiveness is not different from that of CPP-ACP. However, the cariostatic potential of Zamzam water need to be confirmed by further studies before giving any recommendation of using Zamzam water in the dental practice.

REFERENCES
3. Analytical report of Zamzam water cited from the ministry of agriculture and water resources (personal communication) 1971.

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Table 1: Microhardness (Mean* and Standard Deviation) of enamel surfaces treated by Zamzam water, CPP-ACP, and CPP-ACP+NaF after pH Cycling

<table>
<thead>
<tr>
<th>Groups</th>
<th>Zamzam water</th>
<th>Casein phosphopeptides-amorphous calcium phosphate</th>
<th>Casein phosphopeptides-amorphous calcium phosphate+sodium fluoride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>Mean ±S.D</td>
<td>Mean ±S.D</td>
<td>Mean ±S.D</td>
</tr>
<tr>
<td>Sound enamel</td>
<td>263.03 12.59</td>
<td>295.98 16.18</td>
<td>247.81 14.87</td>
</tr>
<tr>
<td>Demineralization</td>
<td>54.93 11.49</td>
<td>57.68 9.02</td>
<td>49.9 6.97</td>
</tr>
<tr>
<td>Remineralization</td>
<td>99.39 8.03</td>
<td>104.66 8.46</td>
<td>129.03 8.51</td>
</tr>
</tbody>
</table>

ANOVA

F= 811.641 F= 921.701 F= 695.834  
P= 0.000 P= 0.000 P= 0.000  
df= 2 df= 2 df= 2

Table 2: LSD test among variables of Microhardness for Zamzam water, CPP-ACP, and CPP-ACP+NaF

<table>
<thead>
<tr>
<th>Groups</th>
<th>Zamzam water</th>
<th>Casein phosphopeptides-amorphous calcium phosphate</th>
<th>Casein phosphopeptides-amorphous calcium phosphate+sodium fluoride</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>Mean difference</td>
<td>P-value</td>
<td>Mean difference</td>
</tr>
<tr>
<td>Sound enamel × Demineralization</td>
<td>208.1 0.000*</td>
<td>238.3</td>
<td>0.000*</td>
</tr>
<tr>
<td>Sound enamel × Remineralization</td>
<td>163.638 0.000*</td>
<td>191.313</td>
<td>0.000*</td>
</tr>
<tr>
<td>Demineralization × Remineralization</td>
<td>-44.463 0.000*</td>
<td>-46.988</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

* Highly significant

Table 3: Microhardness (Mean* Values and Standard Deviation) of enamel surfaces treated by de-ionized water

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ±S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sound enamel</td>
<td>280.85 17.99</td>
</tr>
<tr>
<td>Demineralization</td>
<td>56.63 7.89</td>
</tr>
<tr>
<td>Remineralization</td>
<td>57.29 7.54</td>
</tr>
</tbody>
</table>

ANOVA

F= 905.545  
P= 0.000  
df= 2

Table 4: LSD test among variables of microhardness of enamel surfaces treated by de-ionized water

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sound enamel × Demineralization</td>
<td>224.225</td>
<td>0.000*</td>
</tr>
<tr>
<td>Sound enamel × Remineralization</td>
<td>223.563</td>
<td>0.000*</td>
</tr>
<tr>
<td>Demineralization × Remineralization</td>
<td>-0.662</td>
<td>0.914</td>
</tr>
</tbody>
</table>

* Highly significant

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Figure 1: Mean values of the microhardness of the sound enamel surfaces, after demineralization and following treatment with the selected agents.

Figure 2: Changes in the microhardness values after treatment with the selected agents.