Effect of Zinc Sulfate on Human Primary Enamel Microhardness: An *in vitro* Study

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Objective: *The aim of this study was to assess the effect of zinc sulfate on microhardness of human primary enamel.* ***Method:*** *Sixteen sound primary molars were sectioned mesiodistally. For each sample one of the sections was randomly immersed in artificial saliva (Biotene,USA) and the other was immersed in artificial saliva which was charged with 10 mg/5mL concentration of zinc sulfate supplement (Razak Company, Iran) daily. After 24 days (the expected time to use up one 120-mL bottle) the surface microhardness of enamel was measured by knoop hardness test (KH) with Knoop diamond under a 50-gram load for 10 seconds.* ***Results:*** *The mean (± SD) microhardness values (KHV) for the tested groups were 320 ± 49.45 and 357 ± 36.35, respectively. There were statistically significant differences between the groups as exhibited by independent t-test (P=0.023).* ***Conclusion:*** *Primary teeth immersed in a zinc sulfate rich solution for 24 days showed higher microhardness values in comparison with the control group.*

Keywords: *Zinc sulfate, Enamel, Primary teeth*
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INTRODUCTION

Adequate zinc supply in foodstuffs is critically important in different stages of growth and development. Evidence of human zinc deficiency began to emerge during the 1960s, when cases of zinc responsive dwarfism and delayed sexual maturation were reported for the first time among Egyptian adolescents.[1]

There are countries in South and Southeast Asia, Southern Africa, Central America and the Andean region, which appear to have the highest risk of zinc deficiency and many other countries in these regions are classified as having a moderate risk of zinc deficiency.[2]

There are a variety of extrinsic factors which influence the enamel, and nutritional supplements are one of them; iron, for example, may cause extrinsic staining of human teeth and can also interfere with the dissolution of dental enamel.[3,4]

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The results of some experiments show that remineralization effect can be achieved in the initial stages of caries progression by oral and local application of Zn preparations.[5]

To our knowledge the effect of zinc sulfate on properties of human primary enamel is not clear yet.

The aim of this study was to evaluate the effect of zinc sulfate immersion on microhardness of human primary teeth enamel. The null hypothesis was that there will be no changes in enamel microhardness after 24 days immersion in a zinc sulfate rich solution.

MATERIALS AND METHOD

Sixteen sound human primary molars were selected. The teeth were stored in 0.5% chloramine solution for 24 hours and then immersed in distilled water (Grade 3, ISO 3696). The teeth were sectioned mesiodistally by a low-speed diamond saw cooled by water.

Enamel surfaces of the sections was grounded flat with water-cooled discs (60 to 3000 grades of SiC papers, Matador, Germany) and polished with felt papers with Al2O3 (1µm, Struers, Denmark).

The tooth slices were stored at 4ºC prior to use (according to ISO/TC 11405). For each sample one of the sections was randomly immersed in artificial saliva (Biotene, USA) and the other section was immersed in artificial saliva which was charged with zinc sulfate (Razak Company, Iran), the employed concentration was 10 mg/5 mL(case group).

Since the recommended intake of zinc in children aged 1-6 years is 10 mg/day,[2,6] in the case group the solution was charged with 10 mg of zinc per day for 24 days (the expected time to use up one 120-mL bottle). After application of zinc sulfate, the samples were washed with artificial saliva with a flow rate of 0.5 mL per minute because the salivary flow rate for children aged 1-5 years is estimated to be 7.94 per 15 minutes by considering the following equation[7]:

$$5.6 + (0.78 \times \text{age}) = \text{salivary flow rate in 15 minutes}$$

The surface microhardness of the enamel was measured using a microhardness tester (Five HMV 2000, Shimadzu Corporation, Tokyo, Japan) with a Knoop diamond under a load of 50 g /10 s.

Three indentations were made on each specimen, and the average values were calculated. The average values for the case and control sections of each tooth were compared by independent t-test.

RESULTS

The minimum and maximum microhardness values (KHV) for the teeth which were immersed in artificial saliva were 231.67 and 394.67 respectively and they were 298.33 and 407 for the teeth immersed in rich zinc sulfate solution.

Mean microhardness (±SD) values are reported in Table 1. There was normal distribution by one-sample Kolmogorov-Smirnov test. Independent *t*-test revealed statistically significant differences between the two groups (P=0.023).

Table 1. Enamel microhardness (Kg force/mm^2) in tested groups

GROUP	N	Minimum	Maximum	Mean	Std. Deviation
Artificial saliva	16	231.67	394.67	320.2292	49.45192
Zinc sulfate solution	16	298.33	407.00	357.0625	36.35610

DISCUSSION

This experimental study evaluated the effect of zinc sulfate immersion on microhardness of primary enamel. The findings revealed increased enamel microhardness values after storage in a rich zinc sulfate solution.

Enamel is the hardest tissue in the body and hardly contains collagen; it contains 97 wt% of hydroxyapatite (HA) crystals.[8]

Looking at the HA chemical formula, $Ca_{10}(PO_4)_6(OH)_2$, a single unit cells comprises of 44 atoms that are well-organized in a hexagonal system.[8]

The HA structure allows the incorporation of a wide range of different ionic substitutions, which leads to different degrees of variations in the original HA structure, depending on the type of substitution and the substitution quantity.[9,10]

The reason for an increase in the microhardness of enamel is not clear but it might be attributed to substitution of Zn in HA structure.

Some research studies have indicated that Zn actually exhibits inhibiting effects on the crystallization of HA[11-13] and substitution of Zn^{2+} for Ca^{2+} in the HA structure is extremely difficult; however, Tripathy *et al* prepared Zn hydroxyapatite with a chemical formulation of $Zn_{10}(PO_4)_6(OH)_2$ and the synthesis of apatite samples with low concentrations of Zn were possibly achieved.[10]

In the present study the samples were incubated in zinc sulfate and slowly rinsed by artificial saliva which contained KCl and NaCl.

When the microhardness test was being carried out, the indentor might have contacted not only the apatite of the enamel but also the formed $ZnCl_2$ layer which has low solubility.[14]

It must be pointed out that the hypothesis above still needs confirmation, which might be achieved through the analysis of the contents of Zn and Ca and other ions on the surface of the enamel using atomic absorption spectroscopy or pontentiometric stripping analysis. Zinc is widely used in dentistry and is a component of toothpastes, mouthwashes, dental amalgams and root filling materials; therefore, zinc is released into the saliva and then the oral fluids transport it into the underlying tooth structures, pulp and soft tissues.[15]

Tschoppe *et al* reported that zinc carbonate nano-hydroxylapatite toothpastes exibit higher remineralization effects compared to aminofluoride toothpastes.[16] In addition, there is evidence that under conditions of cariogenic challenge caries progression is inhibited by the fluoride toothpastes containing triclosan and zinc citrate.[17]

It has been established that remineralization effect can be achieved by Zn preparations.[5] Furthermore, the carious primary teeth contain more Zn as compared to teeth without caries.[8]

Finally, zinc is effective in remineralization process and by taking into account the increased microhardness value of enamel in the present study; it appears that Zn has a protective effect on enamel against cariogenic challenges.

Further studies into the structural changes in enamel are recommended.

CONCLUSION

Zinc sulfate enhances the microhardness of enamel in human primary teeth.

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