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Full Length Research Paper

Evaluation of the effect of nanohydroxyapatite on erosive lesions of the enamel of permanent teeth following exposure to soft beer *in vitro*

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Consumption of carbonated acidic beverages is the main etiological factor in dental erosion. Studies have shown that nanohydroxyapatite has the potential to remineralise primary carious lesions. This experimental study was carried out on 18 permanent teeth. Following measurement of the primary micro hardness value of each tooth, the teeth were immersed in 40 ml soft beer, followed by measurement of secondary micro hardness values. The teeth were then randomly divided into two groups NHAP solution and drinking water, and tertiary micro hardness values were then measured. Data analysis was carried out by paired t test (within groups) and ANOVA (between groups) statistical tests. Secondary micro hardness values of the 18 samples had reduced to 92.5% of the primary values and this was found to be statistically significant (p=0.009). Tertiary micro hardness values of the 9 samples in the tap water group were 99.3% of the secondary values, which was not statistically significant (p= 20.6). The tertiary micro hardness values of the 9 samples in the NHAP group had increased; so that the secondary micro hardness values were equivalent to 98.2% of the tertiary micro hardness values, which was statistically significant (p= 0.012). According to the results of the present study, NHAP has the potential to remineralise erosive enamel lesions caused by exposure to soft beer.

Key words: Erosion, micro hardness, enamel, nanohydroxyapatite.

INTRODUCTION

Dental erosion is defined as the irreversible loss of tooth substance by a chemical process not involving bacteria. The main cause of dental erosion is acid exposure (Neville Bw etal.,2002) ; this may be caused by a sudden increase in consumption of soft drinks, diet cokes and fruit juices, which seems to be more significant compared to the other etiologic factors (Bello and AL- Hammed, 2006).

Considering the deteriorating effects of soft beer beverages on the micro hardness of dental enamel, it is essential to search for a material that induces remineralisation of erosive lesions. Recently, it has become possible to use nanotechnology for preparation of nano-filters and nano-composites. Nanotechnology is defined as the construction of materials, parts and useful systems with nanometer dimensions (in longitudinal scale) and subsequent application of the novel characteristics of this minimal scale technology (Freitas Jr, 2000; Jhaveri and Balaji, 2005). The topic of nanotechnology is one of the most commonly discussed areas amongst the international scientific community (Saravanakumar and Vijayalakshmi, 2006); and nanohydroxyapatite (NHAP) is one of the byproducts of nanotechnology. Hydroxyapatite is a material with high tissue biocompatibility; therefore production of hydroxyapatite crystals in nano-sizes enables higher compatibility with enamel crystals. The results of a large

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Table 1. PH of Behnoush lemon delester, drinking water and NHAP solution.

Evaluated liquid	pH
Behnoush lemon delester	4/02
drinking water	6/67
NHAP solution	6/5

number of studies carried out in recent years have shown that toothpastes and mouthwashes containing NHAP have the potential to remineralise primary carious lesions (Haung et al., 2009). The aim of this *in vitro* study was to investigate the effect of NHAP as an anti-erosive agent on enamel micro hardness of third molar teeth following exposure to a soft beer beverage.

MATERIALS AND METHODS

This *in vitro* study was conducted on 18 unerupted third molar teeth. The teeth were removed surgically, and were clinically examined to ensure that they did not exhibit caries, attrition, micro fracture or hypocalcific changes. During the four months collection period, the teeth were stored in newly purchased glass containers that were filled with tap water collected from central Tehran (region 6) and stored at room temperature. In order to prevent surface changes and contamination during this period, the water inside the glass containers was changed twice per week. At the end of the collection period, the surfaces of the teeth were mechanically polished using a prophylaxis brush and paste (containing pumice without fluoride) in slow speed and air turbine hand pieces rotating in low speeds of between 500 to 1500 rpm.

The teeth were then examined under a steromicroscope (Carton Industries Ltd SCW- E- Thailand) (×40) for absence of any enamel irregularities, microscopic carious lesions and micro fractures; 18 teeth were selected as the study sample. Square shaped labels with a diameter 5 × 5 mm were then attached to the distal surfaces of the teeth, and the remaining surfaces were covered by translucent self-curing acrylic resin; this provided uniform dimensions of the exposed surfaces of all of the teeth irrespective of shape, size or group. During acrylic placement, the samples were placed in cold water in order to prevent dimensional changes induced by the exothermic reaction of the setting acrylic.

In order to enable accurate assessment of enamel micro hardness, the surfaces of the samples were polished using 5,000 grit abrasive paper and water irrigation; so that the resulting smooth enamel surface would be compatible with Vickers micro hardness measurement device (Shimadzu M g5037, Japan). After polishing, the surfaces of the samples were dried, and primary micro hardness values of the teeth were measured using the Vickers device. The most suitable point of force application was selected. A force of 50 g was then applied at three separate points on each sample (in the shape of L) and the micro hardness value of each sample was then calculated and recorded.

After recording the primary micro hardness values, each tooth was immersed for 5 min in 40 ml of Behnoush Lemon Delester (Behnoush, Iran), which was poured into a beaker immediately after opening the bottles. Considering the average daily consumption of soft drinks and an average 20 s duration of contact with the mouth (prior to salivary cleansing), the daily consumption of carbonated soft drinks is 5 min (Kitchens and Owens, 2007). Following immersion, the teeth were removed and secondary micro hardness values were measured using the Vickers device. The teeth were then randomly divided into two groups containing 9 samples as follows:

1.Treatment group: consisting of 9 teeth immersed in NHAP solution (10%) (Nanoshell co.)
2. Control group: consisting of 9 teeth immersed in drinking water collected from central Tehran (Region 6)

It should be noted that the pH values of Behnoush Lemon Delester, drinking water and NHAP solution were measured by a pH meter device (METROHM Ltd, Herisau CH-9101, and Switzerland) prior to use (Table 1).

The surfaces of the samples in the control and experimental groups were subsequently washed for 10 min, followed by tertiary measurement of the micro hardness values of the teeth by a blind examiner (who was not aware of the solution in which each tooth was immersed). The collected data was then statistically analyzed, and changes in micro hardness values of the teeth in each group and between groups were analyzed blindly (by a statistician who was not aware of the solutions used in each group) using paired t test and ANOVA statistical analyses.

Due to the indentation caused by the Vickers device at the primary measurement points, subsequent assessments of the micro hardness values at the same points of impact was not possible, and secondary/tertiary micro hardness values were measured at other points on the teeth. Considering lack of statistical significance in micro hardness values at various points of impact on a tooth, it is possible to assume that the micro hardness of any point is representative of the surface micro hardness of each sample (Haghgoo and Foruzesh, 2010).

RESULTS

The average primary micro hardness value of the 18 samples was 340.24±25.4^2 kgf/mm. This had reduced to 314.67±33.89^2 kgf/mm (secondary micro hardness value) following immersion in Behnoush Lemon Delester; this was equivalent to 92.5% of the primary micro hardness value, and analysis by paired t test showed this is statistically significant ($p = 0.009$) (Table 2).

The average secondary micro hardness value of the 9 samples in the water group was 312.85±36.79^2 kgf/mm; this reduced to 310.81±31.44^2 kgf/mm (tertiary micro hardness value) following immersion in drinking water. This was equivalent to 99.3% of the secondary micro hardness value, and analysis by paired t test showed that this is not statistically significant ($p = 20.6$) (Table 3).

The average secondary micro hardness value of the 9 samples in the NHAP group was 315.18±30.65^2 kgf/mm. This increased to 320.99±24.74^2 kgf/mm (tertiary micro hardness value) following immersion in NHAP solution;

Table 2. Micro hardness value before and after immersion in Behnoush Lemon Delester.

Liquid	Number	primary micro hardness value	secondary micro hardness value
Behnoush Lemon Delester	18	340/24+-25/40	314+-33/89

Table 3. Micro hardness value before and after immersion in drinking water and NHAP.

Liquid	Number	Secondary micro hardness value	Tertiary micro hardness value
Drinking water	9	312/85+-36/79	310/81+-31/44
NHAP	9	315/18+-30/65	320/99+-24/74

therefore the secondary micro hardness value was equivalent to 98.2% of the tertiary micro hardness value, and analysis by paired t test showed that this is statistically significant ($p = 0.012$) .

The results showed that there is a statistically significant reduction in the micro hardness of unerupted permanent third molar teeth following exposure to Behnoush Lemon Delester Drink. The results also showed that there is a statistically significant increase in the micro hardness value of unerupted permanent third molar teeth following exposure to NHAP solution; no significant changes were recorded following exposure to water.

DISCUSSION

Dental erosion involves loss of tooth structure by a chemical process not involving bacteria on the tooth surface (Haung et al., 2009). Despite a reduction in the prevalence and severity of dental erosion in recent decades (in particular in developed countries) (Kitchens and Owens, 2007), a significant increase in other pathological entities such as dental erosion has been observed. Although, numerous studies have been carried out in the field of tooth wear and in particular dental erosion since 1980s (Shaw et al., 1998), there is no consensus regarding the prevalence of dental erosion, and recorded statistics range from 25 to 60%. According to recent studies, the prevalence of dental erosion is increasing (Peterson and Bratthal, 996), and the main etiological factor is believed to be exposure to acids (Haung et al., 2009).

One of the main causes of dental erosion is a sudden increase in the consumption of soft beverages, diet drinks and fruit juices. This appears to be more significant compared to the other factors, as the other etiological factors are only present in specific population groups, whereas consumption of acidic beverages has increased significantly in recent years and become prevalent amongst all age groups. Recent studies on dietary intake have revealed that whilst the amount of water intake in children has decreased, there has been an increase in the consumption of acidic drinks (Brown et al., 2007).

Considering the positive effects of NHAP toothpaste on primary carious lesions (Zhng et al., 2007), recent advances in the application of nanotechnology in various scientific fields and the effects of erosion and soft beer consumption on dental enamel, it appeared necessary to study the effects NHAP on enamel erosive lesions that are caused by the application of soft beer.

The results of the present study showed that a statistically significant increase in the micro hardness of teeth demineralised by soft beer was observed following exposure to NHAP solution. In a laboratory study by Haghgoo, the effects of soft beer and coke on 30 unerupted third molar teeth were assessed; the results indicated that there was a statistically significant decrease in the average primary micro hardness values of teeth following exposure to the above drinks (Haghgoo and Foruzesh, 2010). In a study by Fallahinejad et al. (2007) whereby comparison of pH values of various carbonated beverages was carried out, it was shown that calcium was removed from the surface of dental enamel and enamel micro hardness reduced following exposure to these drinks (Fallahinejad et al., 2007). Considering the above studies, it is apparent that enamel micro hardness is reduced following exposure to acidic beverages.

The results of our study were similar to the previous studies carried out by Haghgoo and Fallahinejad (Haghgoo and Foruzesh, 2010; Fallahinejad et al., 2007) as it was shown that a statistically significant reduction in the micro hardness of teeth was observed following exposure to a soft beer beverage.

In this study, an attempt was made to eliminate all other influencing factors; therefore completely healthy unerupted teeth were selected in order to eliminate variations due to oral and dietary habits of individuals from whom the teeth were extracted. Therefore by removing any possible confounding factors and providing similar circumstances in all of the samples, only the effect of the soft beer drink was assessed. In addition, the softest abrasive paper (5000 grit) was used in order to minimize the inadvertent effects of excess enamel removal. In order to provide similar conditions to every-day circumstances, the temperature of the soft beer solution used in this study was maintained at 9°C (similar to fridge temperature).

The results of the present study indicate that NHAP solution has the ability to remineralise erosive lesions caused by soft beer. In studies carried out by Kim et al. (2007) NHAP was used consecutively in mouthwash, toothpaste and chewing gum (Kim et al., 2007; Jeong et al., 2006; Kjolhede and Gyldenvang, 2009). It was concluded that all of these materials had the potential to remineralise dental enamel, although additional substances were present in all of the above studies that could have a synergistic effect with NHAP (similar to the effect of fluoride in the study by Haung and Xiangcai, 2005). Therefore, NHAP was the only material used in this study in order to allow exclusive assessment and enable evaluation of its positive effects on enamel micro hardness.

In a study by Haung et al. (2008), various concentrations (1, 5, 10 and 15%) of NHAP were investigated, and it was concluded that no difference existed between the 10 and 15% concentrations (Xiangcai, 2005); therefore we decided to use 10% concentration of NHAP in this study. Also, in studies by Haung et al. (2008) the effects of NHAP in 12 day, 10 day and 48 hour time intervals were investigated (Kim et al., 2007; Jeong et al., 2006; Kjolhede and Gyldenvang, 2009; Xiangcai, 2005) . However, as it is difficult to apply the above time intervals to clinical situations, it was decided to assess the effect of NHAP in 10 min time intervals. This allowed adjustment of the experimental conditions to clinical circumstances, by assuming that the majority of patients are able to maintain this solution intra orally for a duration of 10 minutes.

In this study, *in vitro* investigation of the effects of NHAP solution on enamel erosive lesions was carried out. It is recommended that additional in situ studies of the effects of NHAP on enamel erosive lesions are conducted in the future.

Conclusion

According to the results of the present study, it can be concluded that NHAP solution has the potential to remineralise enamel erosive lesions caused by exposure to soft beer beverage.

REFERENCES

Bello LL, AL-Hammed N (2006). Pattern of fluid consumption in a sample of Saudi Arabian adolescents aged 12-13 years. Int. J. Pediatr. Dent., 16(3): 168-173.

Brown CJ, Smith G, Shaw L, Parry J, Smith AJ (2007). The erosive potential of flavored sparking water drinks. Int. J. Pediatr. Dent., 17(2): 86-91.

Fallahinejad GM, Nabavi RS (2007). Comparing the effect of Iranian soft drinks with the standard sample, calcium ion analysis. J . Dent. Tehran Univer. Med. Sci., 20(1): 27-32.

Freitas RA Jr (2000). Nanodentistry. J .Am. Dent .Assoc., 131(11): 1559-1565.

Haghgoo R, Foruzesh TF (2010). *In vitro* evaluation on effect of soft drink and soft bear on enamel microhardness. J. Dent. Shiraz. Univer. Med. Sci., 11(2): 153-160.

Haung SB, Gao SS, Yu HY (2009). Effect of nano- hydroxyapatite concentration on remineralization of initial enamel lesion in vitro. Biomed .Mater., 4(3): 034101.

Jeong SH, Jang SO, Kim KN, Kwon HK, Park YD, Kim BI (2006). Remineralization potential of new toothpaste containing nano-hydroxyapatite. Key .Eng. Mater., 309-311(18): 537-540.

Jhaveri HM, Balaji PR (2005). Nanotechnology: the future of dentistry. J Ind. Pros. Soc., 5(1): 15-17.

Kim MY, Kwon HK, Choi CH, Kim BI (2007). Combined effects of nano-hydroxyapatite and NaF on remineralization of early caries lesions. Key Eng. Mater., 330-332(19): 1347-1350.

Kitchens M, Owens BM (2007). Effect of carbonated beverages, coffee, sport and high energy drinks and bottled water on the *in vitro* erosion characteristics of dental enamel. J. Clin .Pediatr Dent., 31(3): 153-159.

Kjolhede T, Gyldenvang L (2009). Chewing gum comprising hydroxyapatite. Fresh Patents, 1: 424-448.

Neville Bw, Damm D, Allen Gm, Bouquot JE (2002). Oral and Maxillo Facial Pathology, 2nd Ed, New York, Sheefer John; chap, 2: 55-58.

Peterson HG, Bratthal D (1996). The caries decline: a review of reviews .Eur. J. Oral. Sci., 104(4): 436-443.

Shaw L, Smith AJ (1999). Dental erosion -the problem and some practical solutions. Bri. Dent. J., 186(3): 115-118.

Saravanakumar R, Vijayalakshmi R (2006). Nanotechnology in dentistry. Ind. J. Dent. Res., 17(2): 62-65.

Zhng LK, Meng JX, Lixy XC (2007). Remineralization effect of the nano-hydroxylapatite toothpaste on artificial caries. Key Eng. Mater., 330-332(19): 267-270.

Xiangcai M, Xingyi L, Kuilong L (2005). Study of nano- hydroxyapatite on the remineralized teeth. Mater. Sci., 457-479(5): 2423- 2426.