

Volume 2 Issue 9 September 2018

Comparative Evaluation of the Efficacy of Milk vs Oxalate Containing Mouthwash (Listerine[™] Advance Defense Sensitive Mouthwash Fresh Mint) on Occlusion of Dentinal Tubules Using SEM Analysis: An *In Vitro* Study

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Received: July 24, 2018; Published: August 29, 2018

Abstract

Dentinal hypersensitivity is a painful clinical condition, which is most commonly encountered in day to day practice. The prevalence of dentinal hypersensitivity increases drastically post periodontal therapy. To overcome the problem of dentinal hypersensitivity, various desensitization agents are used. The present in vitro study evaluates the ability of milk to overcome dentinal hypersensitivity by assessing tubular occlusion via Scanning Electron microscopy. The study was done on 40 extracted human teeth which were divided randomly to be immersed in study (Milk) and control (oxalate containing mouthwash) group for 10 min twice daily, for 15 days. Thereafter the samples were viewed under scanning electron microscope for tubular occlusion. Almost all the tubules (100%) were partially occluded in the study group; 85% of the tubules in the experimental group were completely obliterated. The results were statistically significant with p value < 0.05. Thus, an alternative can be thought of in the form of milk for dentinal hypersensitivity.

Keywords: Dentinal Hypersensitivity; Milk; Tubular Occlusion; SEM; Root Sensitivity; In Vitro

Abbreviations

DH: Dentinal Hypersensitivity; SEM: Scanning Electron Microscopy

Introduction

Dentinal hypersensitivity (DH) is a painful clinical condition, which is most commonly encountered in day to day practice. "Dentine hypersensitivity is characterized by short, sharp pain arising from exposed dentine in response to stimuli, typically thermal, evaporative, tactile, osmotic or chemical and which cannot be ascribed to any other dental defect or pathology" [1].

The pulpo-dental complex is covered and protected by hard tissues like enamel and cementum. Enamel being the hardest structure in the body, once lost cannot be regenerated or repaired. Cementum on the other hand is less hard, resulting in early and rapid loss when exposed to the outside. Thus, both the protecting tissues when lost, expose the underlying dentine which may cause dentinal hypersensitivity. Scanning electron microscopic (SEM) analysis of "hypersensitive" dentin shows the presence of widely open dentinal tubules [2]. The presence of wide tubules in hypersensitive dentin is consistent with the hydrodynamic theory [1]. This theory, as proposed by Brannstrom (1964) is based on the presence and movement of fluid inside the dentinal tubules. The stimuli which tend to move the fluid away from the pulpo-dental complex produce more pain. These stimuli typically include cooling, drying, evaporation and application of hypertonic chemical substances [3]. It should be noted that not all exposed dentine is sensitive. For sensitivity to occur the thin smear layer covering the widely opened dentinal tubules must be lost/removed [2,4].

To overcome the problem of dentinal hypersensitivity, various desensitization agents are used. Recent in the development of desensitization agents is milk protein casein has been used to develop a remineralising agent named GC Tooth Mousse (Recaldent, GC Corp, Japan). This milk protein casein phosphor-peptide

(CPP) contains phosphoseryl sequences which by attaching with amorphous calcium phosphate (ACP) of teeth forms stabilized CPP-ACP. This stabilized CPP-ACP prevents the dissolution of calcium and phosphate ions and maintains a supersaturated solution of bioavailable calcium and phosphates. It has also been shown that this stabilized CPP-ACP can effectively re-mineralize the subsurface enamel lesions. This re-mineralizing capacity of CPP can also help in prevention and treatment of DH [1,5].

Oxalates, on the other hand can reduce dentinal permeability and occlude dentinal tubules. Thirty percent potassium oxalate had shown a 98% reduction in dentinal permeability [6]. Also, topical application of 3% potassium oxalate reduced DH after periodontal therapy [7]. Oxalates reacts with the calcium ions of dentine and forms calcium oxalate crystals inside the dentinal tubules as well as on the dentinal surface. This results in a better sealing as compared with an intact smear layer [8].

As oxalates and milk both occlude the dentinal tubules, both are considered for comparison in the study.

Aim of the Study

To evaluate and compare in vitro the efficacy of Milk vs oxalate containing mouthwash (ListerineTM Advance Defense Sensitive Mouthwash Fresh Mint) on occlusion of dentinal tubules.

Objective of the study

- To evaluate *in vitro*, the efficacy of Milk vs oxalate containing mouthwash (Listerine[™] Advance Defense Sensitive Mouthwash Fresh Mint) on occlusion of dentinal tubules, on extracted human teeth using SEM analysis.
- To Compare *in vitro*, the efficacy of Milk vs oxalate containing mouthwash (Listerine[™] Advance Defense Sensitive Mouthwash Fresh Mint) on occlusion of dentinal tubules, on extracted human teeth using SEM analysis.

Materials and Methods

Source of data

Considering the difference in group means to be 20%, power of the study as 80%, at 95% confidence interval, a ratio of sample size (group1/group 2) as 1 and with the significance level set at 5%, a sample size of 40 was derived (i.e. 20 in each group), as suggested by the statistician.

40 samples of freshly extracted teeth were obtained and were divided into 2 groups of 20 each.

Inclusion criteria: Intact extracted teeth from human subjects of different ages were obtained and stored in physiologic saline solution.

Exclusion criteria: Carious teeth were excluded from the study.

Study design

A total sample size of 40 extracted human teeth was considered for the *in vitro* study. The teeth were cleaned thoroughly, scaled if necessary to remove any calculus deposits if present and stored in normal saline solution

Group A: (Experimental group): 20 extracted teeth

Each tooth in group A was immersed in 30 ml of Milk for 5 minutes, twice daily at an interval of 12 hours for 15 days. The milk was changed for every immersion. Powdered Milk (1/2 tablespoon) mixed with distilled water (30 ml) was used for the procedure, in order to standardize the solution for every use. The specimens were then viewed under the scanning electron microscope.

Group B: (Control group): 20 extracted teeth

Each tooth in Group B was immersed in 30 ml of oxalate containing mouthwash (Listerine[™] Advance Defense Sensitive Mouthwash Fresh Mint) for 5 minutes twice daily at an interval of 12 hours for 15 days. The mouthwash was changed after every use. The oxalate containing mouthwash is commercially available as Listerine[™] Advance Defense Sensitive Mouthwash Fresh Mint, hence standardization was possible. The specimens will then be viewed under the scanning electron microscope.

Results and Discussion

Each specimen was photo-micrographed. The photomicrographs were evaluated by the principle investigator and ranked according to a graduated scale expressing the relative openness (or occlusion) of the dentinal tubule orifices. The following criteria were used to rank the photomicrographs:

- Score 0 = No obliteration of tubules
- Score 1 = Slight obliteration of some tubules
- Score 2 = Some obliteration of most tubules
- Score 3 = Complete obliteration of all tubules

Data collected was entered into a computer and analyzed using the SPSS software. Descriptive and inferential statistical analyses were carried out in the present study. Results on continuous measurements were presented on Mean ± SD and results on categorical measurements were presented in Number (%). Level of signifi-

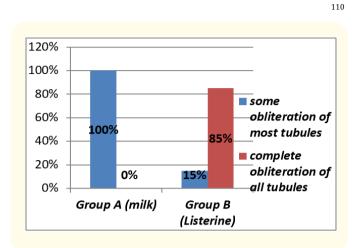
Citation: Nikita Patil, et al. "Comparative Evaluation of the Efficacy of Milk vs Oxalate Containing Mouthwash (Listerine[™] Advance Defense Sensitive Mouthwash Fresh Mint) on Occlusion of Dentinal Tubules Using SEM Analysis: An *In Vitro* Study". Acta Scientific Dental Science 2.9 (2018): 108-112.

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cance was fixed at p = 0.05 and any value less than or equal to 0.05 was considered to be statistically significant.

Chi square analysis was used to find the significance of study parameters on categorical scale. Student t test (two tailed, independent) was used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters.

- Group A showed some obliteration of most tubules, thus a Score 2 was assigned
- Group B showed complete obliteration of tubules in 85% of samples, a Score 3 was assigned. And remaining 15% sowed some obliteration of most tubules- Score 2 was assigned.



			Dentinal tubule orifices		Total
			Some obliteration of some tubules	Complete obliteration of most tubules	
Group	Group A	Count	20	0	20
		% within Group	100.0%	0.0%	100.0%
	Group B	Count	3	17	20
		% within Group	15.0%	85.0%	100.0%
Total		Count	23	17	40
% within Group		57.5%	42.5%	100.0%	
		Chi square value	: 29.565 P value: < 0.0	5**	

Table 1: Comparison of the openness (or occlusion) of dentinal tubule orifices among both the groups using chi square test. $(p < 0.05 - Significant^*, p < 0.001 - Highly significant^**).$

Group A, showed some obliteration of dentinal tubules, however no tubule showed complete obliteration. Group B on the other hand had maximum number (85%) of completely obliterated dentinal tubules. The intergroup analysis shows statistically significant results with p value < 0.05.

Discussion

The common sequelae of periodontal therapy is dentinal hypersensitivity which may persist for a long duration. Discomfort and sometimes pain caused by DH may refrain a person from establishing and maintaining adequate oral hygiene, which further may complicate oral health. Also, once the overlying protective structure is lost, the underlying dentine abrades 25 times faster thus deteriorating the condition more further [9].

B Von Troil found a prevalence of root sensitivity of 9 - 23% and 54 - 55% before and after periodontal therapy respectively and proposed that it occurs in approximately half of the patients

following subgingival scaling and root planing and its intensity increases for a few weeks after therapy and decreases afterwards [10,11].

DG Gillam and R Orchardson stated that the individuals with periodontal disease have higher prevalence of dentinal hypersensitivity after periodontal therapy such as scaling and root planing procedures as compared to those presenting with healthy mouths and gingival recession [12]. Tammaro., *et al.* concluded that successful periodontal treatment can be accomplished through good oral hygiene self-care measures, and by nonsurgical and surgical periodontal therapies which have unwanted side effects including gingival recession, exposure of underlying dentine following root cementum denudation with the risk of experiencing tooth sensitivity [13].

The purpose of this study was to assess the efficacy of milk to occlude dentinal tubules through SEM analysis. The experimental

group (Group A) showed some obliteration of most of the tubules (Score 2). The control group (Group B) on the other hand showed complete obliteration of all the tubules (Score 3) in 85% of the samples assessed (Figure 1).

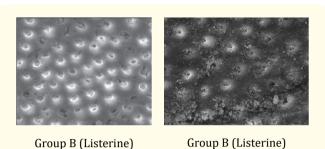


Figure 1: Scanning electron microscopy images.

The statistical analysis thus reveal the study to be statistically significant, with the p value < 0.05 (Table 1).

Milk is a mixture of emulsions, colloids, molecular and ionic solutions. Fresh milk has a pH of 6.7 and is slightly acidic. Milk is also an excellent buffering solution which can resist a change in pH on addition of acid or alkali. When the pH of milk is changed, the acidic or the basic groups of the milk proteins will be neutralized. With the fall of the pH of milk, the charge on the casein also falls and it precipitates. Milk comprises less than 1% salts mainly in the form of chlorides, phosphates, citrates of calcium, sodium and magnesium. Calcium, magnesium, phosphorus and citrates are distributed between the soluble and colloidal phases. Equilibria of salts in milk are altered by heating, cooling and change in pH. Milk also contains various enzymes like phosphatases, lipases, peroxidases and catalases etc [14].

Conclusion

Milk is a suitable, cheaper, fast acting, home-use and easily available solution to the problem of dentine hypersensitivity and can be used as desensitizing agent for dentine hypersensitivity due to periodontal treatment procedures. The present study was an in vitro study assessing occlusion of dentinal tubules after immersion in 30 ml milk for 5 minutes, twice daily for a period of 15 days. It clearly demonstrates the effectiveness of milk for the same. Nevertheless, further studies are required to know the mechanism of milk rinse in prevention of dentine hypersensitivity.

Conflict of Interest

None.

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