

# Comparison of salivary pH changes with tap water and mineral water rinse after 50% sucrose solution rinse - A crossover trial

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## ABSTRACT

Oral hygiene is the primordial level of prevention of dental caries. Since mouth rinsing habit is a daily oral hygiene habit, modification with pH modifying agents can greatly help in the prevention of dental caries. The aim of the study was to compare the effect on salivary pH following mouth rinse using tap water and various brands of mineral water after rinsing with 50% sucrose solution. A crossover trial was done among 60 children in the age group of 3-6 years. The salivary pH was measured at three intervals at baseline, after rinsing with 50% sucrose solution and after rinsing with tap water. After 30 min, the similar protocol is followed for measuring pH after rinsing with mineral water. Randomization was followed in selecting the brand of mineral water to be used. The results obtained were compared with Wilcoxon Signed ranks test the salivary pH after tap water and mineral water rinse and with Kruskal–Wallis Test in between the three brands of mineral water. There was a significant difference in salivary pH after the tap water and mineral water rinse with a higher pH after mineral water rinse. However, there was no significant difference in between the three different brands of mineral water. Rinsing mouth with a solution having higher alkaline pH leads to neutralization of acid production, thereby preventing the caries process.

**Keywords:** Demineralization, mineral water, mouth rinse, salivary pH, tap water

## Introduction

Although preventable, dental caries is considered to be the most common condition of childhood.<sup>[1]</sup> Consumption of more sugary food<sup>[2]</sup> and less frequency of oral hygiene practice<sup>[3]</sup> are a major determinant of the same. Early childhood caries, a more common condition of childhood is a multifactorial disorder with improper dietary pattern and oral hygiene habits being a major determinant. Children experiencing caries in the early childhood phase are more of development of caries in the permanent dentition.

Caries cannot only be prevented by controlling dietary habits, but also by optimal oral hygiene habits<sup>[4]</sup> Studies have shown that children with good oral hygiene practice have a lower incidence of caries than children with poor oral hygiene practice.<sup>[5,6]</sup>

Brushing and flossing are the oral hygiene practices most commonly practiced in daily life.<sup>[7]</sup> Tooth brushing with a fluoridated toothpaste

is effective in reducing the prevalence of caries.<sup>[6]</sup> Flossing tends to remove plaque from the proximal tooth surface, thereby preventing dental caries.<sup>[6]</sup> The method of cleaning and frequency is important determinants in caries prevention.<sup>[7]</sup> Mouth rinsing with fluoride mouth rinses was a mass prophylactic measure in the prevention of dental caries as recommended by FDI (expansion).<sup>[8]</sup> Another type of mouth rinses such as chlorhexidine mouth rinse<sup>[9]</sup> and salt water mouth rinse<sup>[10]</sup> also aids in caries prevention.

According to Key's triad, salivary bacteria play an important role in the development of carries along with dietary habit (substrate) and host.<sup>[4]</sup> The oral biofilm mainly consists of predominant bacterial species from coccoid or straight rod bacteria.<sup>[11]</sup> The role of biofilm has a major role in the caries initiation and progression. *Streptococcus mutans*, *Enterococcus faecium*, *Aerococcus viridans*, *Actinomyces meyeri*, *Lactobacillus acidophilus*, and *Eubacterium limosum* isolated from oral biofilm are known have increased titers in the presence of caries.<sup>[12]</sup> Study by Santos *et al.* have shown a relationship between the frequency and type of oral hygiene practice on the oral biofilm. Oral hygiene practice has shown to decrease the accumulation of biofilm, indirectly halting the caries process.<sup>[13]</sup> Salivary pH is another important determinant in causation of carries. Hence, modification of salivary pH to alkalinity plays an important role in the prevention of dental caries.<sup>[14]</sup> Increase in the clearance rate changes the salivary pH therapy modifying the pH of the plaque and promoting remineralization.<sup>[15]</sup> For relative protection against dental caries, flow rate, buffering capacity, calcium phosphate, and fluoride concentration in the saliva are essential.<sup>[14]</sup>

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According to Watt in 2005, people tend to imply oral hygiene practice that takes less time and effort. With this purpose, a crossover trial was conducted in Chennai to evaluate and compare the effect on salivary pH after tap water and mineral water mouth rinse following rinsing with 50% sucrose.

## Methodology

A crossover randomized control trial was done in preschool children. The study design protocol and informed consent were approved by the Ethical Committee board of the institution with the ethical number STP/SDMDS16PED3. The procedure was followed in accordance with the Helsinki Declaration of 1975 that was revised in 2000. The inclusion criteria were that children should be between 2 and 6 years of age, they were medically healthy, had full primary dentition with or without the eruption of first permanent molar and the parent or guardian provided informed consent for the child's participation. Children with intraoral or extraoral abscess and parents who were not willing to participate in the study were excluded. These children were enrolled from the preschools in Chennai as well as randomly selected patients visiting Saveetha Dental College and Hospital for dental treatment. Based on the sample size calculated block randomization was followed.

A pre-screening was done to obtain a dmft score for each patient. This was done to include an equal number of caries-free children and children with ECC for comparison of the result between the two groups.

## Sample size calculation

The sample size was calculated by a pilot study done among 15 participants with all the participants rinsing with tap water after 50% sucrose solution, and 5 participants rinsing with the individual brand of mineral water. The sample size calculation was done with G power using *a priori* where the difference between two dependent matched pairs was compared. The  $\alpha$  error was taken as 0.05 and  $1-\beta$  as 0.95 with the effect size of 0.5574. The sample size obtained was 44 in total. This sample size was overestimated to compensate for attrition and salivary sample was collected from 60 preschool children.

## Data collection

All children were asked to swish mouth with tap water and any one of the three brands of mineral water (Bisleri, Aquafina, Kinley) after a 50% sugar rinse. The three different brands of mineral water were maintained confidential to the patients by labeling them as A, B, and C, respectively. The observer and patient blinding was thus carried out. The three brands of mineral water were checked for their brands after the completion of the study and were labeled as A, B, and C throughout the study. In the first attempt, unstimulated saliva samples were taken, and pH was measured. After this, all participants were asked to swish and swift a 50% sugar solution for 1 min. 15 min later saliva sample is obtained, and pH is measured by electrical pH meter. Immediately after this, they rinsed the mouth with tap water for 1 min. A third sample was taken after another 15 min and pH was measured. Based on the block randomization, the chit allocation method was

followed where the brand of mineral water to be given as chosen. After 30 min, again unstimulated saliva was taken after which, all participants are asked to swish with 50% sugar solution for 1 min and saliva sample was collect after 15min and pH measured. Immediately after this, they rinsed the mouth with any one of the three brands of mineral water for 1 min and third saliva sample was obtained after 15 min and pH was measured to avoid diurnal variation of the pH, all the samples were collected during the morning time in between 9 am and 12 pm.

pH data recorded were grouped as pre pH, pH after 50% sucrose rinse, pH after tap water rinse, and pH after individual mineral water rinse. The change in the pH of saliva after tap water rinse and mineral water rinse were compared. As well change in pH after using various brands of mineral water were also analyzed In addition, the difference in the pH between caries free and children with ECC was compared.

## Statistical analysis

The data obtained were analyzed using SPSS software version 22. The normality tests Kolmogorov–Smirnov and Shapiro–Wilks tests results reveal that data obtained from individual brand of mineral water did not follow normal distribution. Wilcoxon signed ranks test was used to compare pH levels between tap water and mineral water rinse. Kruskal–Wallis test was used to compare pH levels between the three brands of mineral water. Significance level is fixed at 5% ( $\alpha = 0.05$ ). The drop in the pH after the 50% sucrose rinse and rinse in pH after tap water rinse between caries-free children and children with ECC were compared using Mann–Whitney's test ( $P < 0.05$ ).

## Results

Of the total children recruited in the study, 45% were boys and 55% were girls [Table 1] in the age group of 3-6 years with the mean age of 4 years and 9 months. 13.3% belong to the 3 years age group, 20% belong to 4 years, 30% belong to 5 years, and 36.7% belong to 6 years age group [Table 2].

The mean pH of the saliva after tap water rinse in caries-free children was 8.25 (standard deviation [SD] - 0.276) and median (interquartile range) of 8.20 (8.30-8.40). The mean pH of saliva after tap water rinse in children with ECC was 8.34 (SD-0.925) and median (interquartile range) of 8.30 (8.00-8.40). The mean pH of the saliva after mineral water rinse in caries-free children was 8.63 (SD-0.229) and median (interquartile range) of 8.60 (8.50-8.80). The mean pH of saliva after mineral water rinse in children with ECC was 8.83 (SD-0.950) and median (interquartile range) of 8.60 (8.40-8.80) [Table 3].

Comparative analysis of Wilcoxon signed rank test [Table 4] [Figure 1] reveal that salivary pH is higher after rinsing with mineral water as compared to salivary pH after rinsing with tap water ( $P < 0.001$ ).

Table 1: Frequency table for gender

Gender	n (%)
Male	27 (45.0)
Female	33 (55.0)
Total	60 (100.0)

The mean (SD) pH after rinsing with individual A or B or C brand of mineral water is 8.76 (0.685), 8.82 (0.742), and 8.61 (0.666) having a median (interquartile range) value of 8.60 (8.50-8.75), 8.65 (8.55-8.85), and 8.60 (8.40-8.85), respectively [Table 5].

Comparative analysis of Kruskal–Wallis test [Table 6 and Figure 2] reveals no significant variation in the salivary pH after rinsing with the three different brands of mineral water ( $P=0.609$ ).

According to Mann–Whitney’s [Tables 7 and 8 and Figure 3] test, there was a significantly more increase in the salivary pH after tap water

**Table 2: Descriptive statistics for age**

Statistic	Value
<i>n</i>	60
Mean	4.90
SD	1.053
Minimum	3.0
Maximum	6.0
1 <sup>st</sup> quartile	4.0
Median	5.0
3 <sup>rd</sup> quartile	6.0

SD: Standard deviation

Age	<i>n</i> (%)
3	8 (13.3)
4	12 (20.0)
5	18 (30.0)
6	22 (36.7)
Total	60 (100.0)

**Table 3: Descriptive statistics**

Type of water	DMFS	
	Absent	Present
Tap water		
<i>n</i>	30	30
Mean	8.25	8.34
SD	0.276	0.925
Median	8.30	8.30
1 <sup>st</sup> quartile	8.20	8.00
3 <sup>rd</sup> quartile	8.40	8.40
Mineral water		
<i>n</i>	30	30
Mean	8.63	8.83
SD	0.229	0.950
Median	8.60	8.60
1 <sup>st</sup> quartile	8.50	8.40
3 <sup>rd</sup> quartile	8.80	8.80

SD: Standard deviation

**Table 4: Wilcoxon signed ranks test to compare pH levels between tap water and medication**

Type of water	Ranks	<i>n</i>	Mean rank	Z-value	P-value
Mineral water - tap water	Negative	1	58.00	6.267	<0.001
	Positive	58	29.52		

rinse in ECC as compared to caries-free children. Similarly, there was more decrease in the salivary pH in ECC group as compared to caries free group [Figure 4].

The range of pH change is higher for children with ECC as compared to carried free children. The fall of the pH below the critical pH is more in children with ECC as compared to caries free group.

**Table 5: Descriptive statistics**

Change in pH levels after mineral water	Group		
	Agent A	Agent B	Agent C
PH levels after mineral water			
<i>n</i>	20	20	20
Mean	8.76	8.82	8.61
SD	0.685	0.742	0.666
Median	8.60	8.65	8.60
1 <sup>st</sup> quartile	8.50	8.55	8.40
3 <sup>rd</sup> quartile	8.75	8.85	8.85

**Table 6: Kruskal–Wallis test to compare pH levels between various brands of mineral water**

Various brands of mineral water	Group	<i>n</i>	Mean rank	Chi-square value	P-value
Mineral water	Agent A	20	29.43	0.991	0.609
	Agent B	20	33.60		
	Agent C	20	28.48		

**Table 7: Descriptive statistics**

Variables in tap water group	DMFS	
	Absent	Present
Increase after tap water rinse from sugar		
<i>n</i>	30	30
Mean	0.64	1.26
SD	0.425	1.461
Median	0.60	0.60
1 <sup>st</sup> quartile	0.50	0.50
3 <sup>rd</sup> quartile	0.60	1.00
Percentage increase after tap water rinse from sugar		
<i>n</i>	30	30
Mean	8.80	20.56
SD	7.119	26.799
Median	7.64	7.74
1 <sup>st</sup> quartile	6.25	6.33
3 <sup>rd</sup> quartile	8.00	15.63
Decrease after sugar from baseline		
<i>n</i>	30	30
Mean	0.63	1.18
SD	0.397	1.379
Median	0.50	0.60
1 <sup>st</sup> quartile	0.40	0.40
3 <sup>rd</sup> quartile	0.70	0.80
Percentage decrease after sugar from baseline		
<i>n</i>	30	30
Mean	7.61	13.33
SD	4.826	13.459
Median	6.17	7.14
1 <sup>st</sup> quartile	4.88	5.00
3 <sup>rd</sup> quartile	8.24	11.27

SD: Standard deviation, DMFS: Decay-missing-filled surfaces

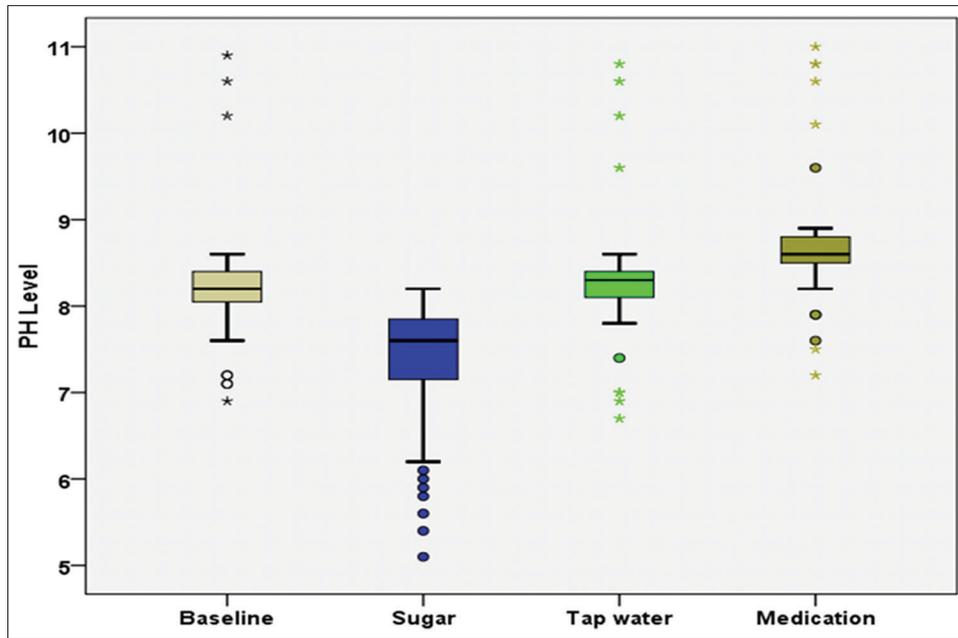


Figure 1: Comparison between tap water and mineral water. (Mineral water named medication for the purpose of blinding for data analysis)

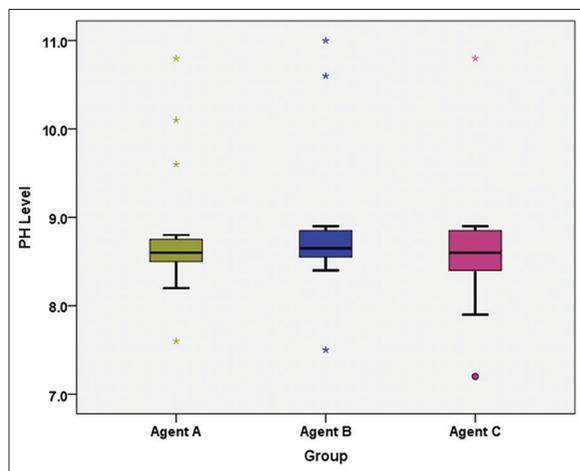


Figure 2: Comparison of the three brands of mineral water (Agent A: Bisleri, Agent B: Aquafina, Agent B: Kinley)

## Discussion

Salivary pH is an important salivary parameter affecting the carious process.<sup>[16]</sup> Demineralization and remineralization happens depends on the pH of saliva. The alkaline pH of saliva neutralizes the acid produced by the plaque bacteria.<sup>[16,17]</sup> Further, a more basic pH of saliva aids in remineralization by precipitation of bicarbonate ions.

Stephan's curve describes increased demineralization on repeated exposure to low salivary pH.<sup>[18]</sup> Stephan reported that plaque pH fall after sugar exposure was less in caries-free individual as compared to children with caries.<sup>[19]</sup> According to Leach, enamel solubility is largely dependent on the plaque pH and the acid production leading to fall in pH.<sup>[20]</sup> Agus *et al.*<sup>[21]</sup> and Van Houte *et al.*<sup>[22]</sup> have also done similar

Table 8: Mann–Whitney test to compare the differences in pH levels between DMFS groups

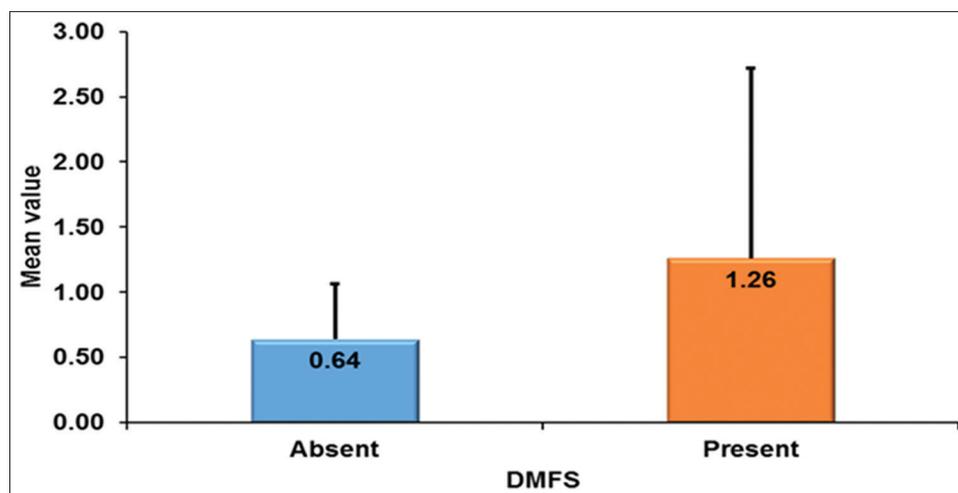
Changes	DMFS	n	Mean rank	Z-value	P-value
Increase after tap water rinse from sugar	Absent	30	28.23	1.020	0.308
	Present	30	32.77		
Percentage increase after tap water rinse from sugar	Absent	30	27.50	1.332	0.183
	Present	30	33.50		
Decrease after sugar from baseline	Absent	30	28.25	1.009	0.313
	Present	30	32.75		
Percentage decrease after sugar from baseline	Absent	30	27.55	1.309	0.190
	Present	30	33.45		

DMFS: Decay-missing-filled surfaces

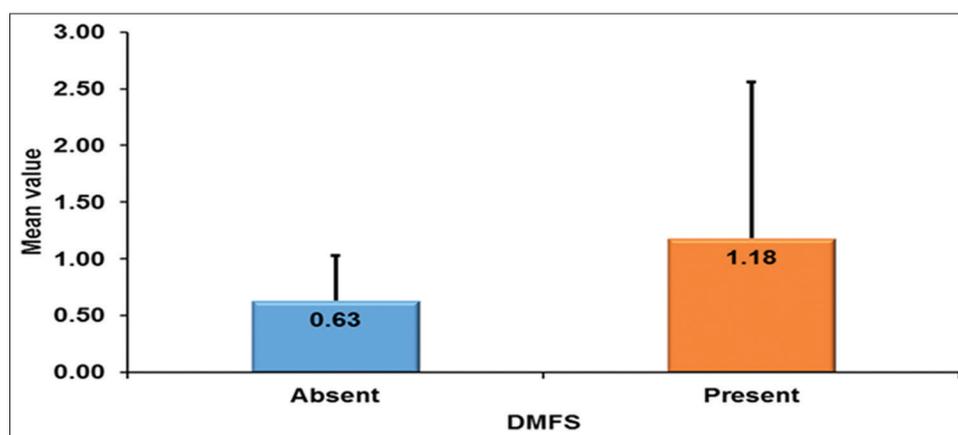
studies in animal and *in vitro* showing the decrease in the salivary pH as a determinant of dental caries.

This study shows a significantly greater rise in salivary pH with tap water and mineral water rinse from the pH after 50% sucrose solution. Rinsing with water thus results in dilution of the acid produced after the sugar consumption thereby causing neutralization and rise in pH. Study by Lim *et al.* have concluded that pH of the saliva increase if the mouth rinse is done with a solution having alkaline pH.<sup>[23]</sup> Another study contradicted the fact and stated that tap water rinsing has no significant effect on the salivary pH and showed xylitol chewing gum having higher effect.<sup>[17]</sup>

The composition of mineral water shows a more alkaline pH as compared to tap water which thereby helps in more neutralization of the acid produced thereby raising the pH more as compared to tap water as found in this study.<sup>[23]</sup> However, no other study has been reported that has compared buffering capacity of saliva with a



**Figure 3:** Increase in pH after tap water rinse from sugar in ECC (present) and caries-free groups (absent)



**Figure 4:** Decrease in pH after sucrose rinse in between ECC (present) and caries-free groups (absent)

solution of different alkalinity. A study has stated that rinsing mouth with mouthwash being more alkaline aids in neutralizing the acidity, which can be indirectly correlated to this study.<sup>[16]</sup>

There were no statistically significant differences in the change in pH of salivary in between the three different brands of mineral water. The presence of increased bicarbonate in the saliva leads to a supersaturated solution thereby increasing the pH.<sup>[24]</sup> The clearance rate and buffering capacity of saliva are a major determinant in the carious process.<sup>[16]</sup> Gopinath *et al.* stated that the clearance rate and the quantity of the secretion of saliva change the pH. In this study, the protective effect of tap water rinse can be attributed to increases in the clearance rate, thereby altering the pH having a more protective effect toward caries prevention. Thus, altering buffering capacity of saliva by altering salivary pH can aid as a preventive measure. Previous studies have also stated that salivary buffering capacity is an important determinant in caries progression and prevention.<sup>[10]</sup> This finding correlated with the previous study by Johansen *et al.* telling about the lower buffering capacity of saliva makes it more caries prone.<sup>[25]</sup> According to this study, tap water rinse provide increase buffering capacity of saliva therapy aiding as a preventive factor. The

present study shows an increase in salivary pH with mineral water as compared to tap water which can be attributed to the higher pH of the mineral water as compared to tap water, thus increasing the buffering capacity of saliva.<sup>[23,26,27]</sup> The present study does not show a significant difference in between the three brands of mineral water. This can be attributed to a less variation in between the pH of individual mineral water leading to insignificant changes.

The present study also shows a significantly higher change in the salivary pH in children with ECC as compared to caries-free children. A similar finding has been noted by Stephan in his study<sup>[19]</sup> which noted a higher fall in pH after sugar consumption in children with ECC.

The present study, however, does not explain the molecular level phenomenon involved in the plaque pH changes. Hence, further studies need to be carried out to study the molecular level changes occurring with the use of solutions having increased buffering capacity. Furthermore, further studies need to be carried out to evaluate the influence of the rinsing and oral hygiene practice on the individual composition of the saliva to determine the correct causation relationship.

## Conclusion

Water rinsing increases salivary pH after the reduction with 50% sucrose solution. Rinsing with a solution that is mineral water having a more alkaline pH leads to increase in salivary pH, which thereby aids as a stop in the process of demineralization by acid production. This indirectly enhances the caries prevention and a simple oral hygiene practice which can be easily used.

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