Comparative Evaluation of 0.2 percent Chlorhexidine and Magnetized Water as a Mouth Rinse on Strepococcus mutans in Children

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ORIGINAL ARTICLE

INTRODUCTION
The removal of supragingival and subgingival bacterial biofilm is a decisive component in the prevention and treatment of dental caries and periodontal diseases.

The microorganisms in bacterial plaque cause inflammatory periodontal disease. For, this reason plaque control plays a significant role in the prevention of caries, gingivitis and periodontitis. Both mechanical procedures and local chemotherapeutics (Cummins 1997)1 are used for this purpose.

Chlorhexidine gluconate, a cationic bis-biguanide was introduced for human use in 1957 in Great Britain.

Chlorhexidine (0.2%) mouthrinse has also shown antibacterial efficacy. C Rindom, WW Briner and H Loe (1976)2 found a reduction of 30 to 50% in the population of S. mutans after rinsing with 10 ml of 0.2% chlorhexidine mouthrinse once daily.

Magnetism is well known in the field of physics. Magnets prove to be strong safeguard against illness and serve as a highly beneficial preventive device. When water passes through the magnetic field, it undergoes certain changes. The magnetic field alters the electrical characteristics of hydrogen ions as well as minerals.

The force of magnetism has a great influence on living organism. When a permanent magnet is kept in continuous contact with water, for considerable time, the water is not only influenced by the magnetic flux of magnet, but also becomes magnetized and acquires magnetic properties. Best results are achieved when water is magnetically treated just prior to use.3 Since many researches have been done with the use of magnets in medical field, its use in dentistry is still lacking.

AIMS AND OBJECTIVES
1. To evaluate and compare antibacterial efficacy of commercially available 0.2% chlorhexidine mouth rinse and conventionally prepared magnetized water on S. mutans.
2. To compare and evaluate that dosage, frequency and duration of use of 0.2% chlorhexidine mouth rinse and magnetized water have any effect on colony count of S. mutans.
MATERIALS AND METHODS

This study was conducted in the year 2007-2008 at Arya Orphanage, Pataudi House, Darya Ganj, New Delhi.

Selection Criteria
Total sample size of 50 children was selected between the age group of 5 to 12 years. The study was conducted over a period of 1 week.

Subject Selection Criteria
- Systemically healthy patients
- No fixed or removable orthodontic appliances or removable prosthesis
- No history of antibiotic therapy in the subjects within previous 3 months
- No use of chlorhexidine mouth wash or magnetized water as oral rinse earlier
- No history of oral prophylaxis done for at least 3 months prior to study.

After selection oral prophylaxis of all the subjects was done using ultrasonic scaler. Then the subjects were instructed to abstain from any oral hygiene measures for next 24 hours.

Baseline saliva sample was collected by spitting method in sterile sample collecting bottles for all the subjects.

Subjects were then divided into three major groups (Flow Chart 1).

Method of Magnetizing Water
RO water was taken in glass bottles and was kept over the magnets for 24 and 72 hours for magnetization.

To check for the magnetization, of the 3 samples (RO water, RO water 24 hours magnetized, RO water 72 hours magnetized) were sent to ‘Metropolis laboratory’ to check for pH and electrical conductivity which reported as follows:

<table>
<thead>
<tr>
<th>Type of water</th>
<th>pH</th>
<th>Electrical conductivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RO water—normal</td>
<td>7.2</td>
<td>25.1</td>
</tr>
<tr>
<td>Magnetized water—24 hours</td>
<td>7.5</td>
<td>24.8</td>
</tr>
<tr>
<td>Magnetized water—72 hours</td>
<td>7.98</td>
<td>11</td>
</tr>
</tbody>
</table>

Days of Sample Collection

For Saliva
- Day 1—baseline, morning and evening
- Day 4—evening
- Day 7—morning and evening.

The samples were collected in sterile sample bottles to check for the S. mutans count and were carried in the ice box containing ice (as transport media) to microbiology laboratory where the culture plates were inoculated for the S. mutans count.

Mutans Sanguis Agar: Himedia
This agar is recommended for differentiation of S. mutans and S. sanguis associated with oral microflora.

S. mutans forms rough, heaped, irregular colonies resembling frosted glass. Mostly crumbly which are white, gray or yellow in color and 0.5 and 2 mm in diameter.

RESULTS
Table 1 shows the mean and standard deviation values at various levels of all the groups. Tables 2 to 6 show a student t-test to compare the differences at various levels of all the groups.

DISCUSSION
The surface of the oral cavity is constantly colonized by microorganisms. One milliliter of whole saliva may contain more than 200 million organism representing more than 250 different species.

Streptococcus constitutes an essential part of the microflora which constantly colonize the mucous membrane and the teeth. The streptococci in the oral cavity comprise S. sanguis, S. mitis, S. salivarius, S. intermedius and other streptococci of which mutans streptococci especially S. mutans and S. sobrinus are maximum.
### Table 1: Mean and standard deviation values of *S. mutans* (in cfu/ml) at various levels of groups I, IIA, IIB and III

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline</th>
<th>1st day morning</th>
<th>1st day evening</th>
<th>4th day evening</th>
<th>7th day morning</th>
<th>7th day evening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>140.25 ± 48.02</td>
<td>2.75 ± 10.86</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Group IIA</td>
<td>117.50 ± 37.16</td>
<td>108.50 ± 39.69</td>
<td>103.50 ± 29.75</td>
<td>89.00 ± 24.98</td>
<td>82.50 ± 29.00</td>
<td>70.50 ± 32.59</td>
</tr>
<tr>
<td>Group IIB</td>
<td>165.00 ± 45.00</td>
<td>144.50 ± 48.70</td>
<td>120.50 ± 39.90</td>
<td>88.00 ± 24.82</td>
<td>71.50 ± 16.29</td>
<td>64.50 ± 22.07</td>
</tr>
<tr>
<td>Group III</td>
<td>160.00 ± 43.59</td>
<td>160.00 ± 43.59</td>
<td>97.50 ± 30.52</td>
<td>65.00 ± 16.58</td>
<td>39.00 ± 16.25</td>
<td>30.00 ± 10.00</td>
</tr>
</tbody>
</table>

### Table 2: Statistical comparison (by unpaired t-test) of *S. mutans* (in n × 10³ cfu/ml) of mean change at various levels between groups I and IIA

<table>
<thead>
<tr>
<th>Groups</th>
<th>1st day morning</th>
<th>1st day evening</th>
<th>4th day evening</th>
<th>7th day morning</th>
<th>7th day evening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>137.50 ± 53.70</td>
<td>140.25 ± 49.27</td>
<td>140.25 ± 49.27</td>
<td>140.25 ± 49.27</td>
<td>140.25 ± 49.27</td>
</tr>
<tr>
<td>Group IIA</td>
<td>9.00 ± 16.80</td>
<td>14.00 ± 20.79</td>
<td>28.50 ± 33.25</td>
<td>35.00 ± 37.78</td>
<td>47.00 ± 39.59</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Significance</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
</tr>
</tbody>
</table>

HS – highly significant

### Table 3: Statistical comparison (by unpaired t-test) of *S. mutans* (in cfu/ml) of mean change at various levels between groups I and IIB

<table>
<thead>
<tr>
<th>Groups</th>
<th>1st day morning</th>
<th>1st day evening</th>
<th>4th day evening</th>
<th>7th day morning</th>
<th>7th day evening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>137.50 ± 53.70</td>
<td>140.25 ± 49.27</td>
<td>140.25 ± 49.27</td>
<td>140.25 ± 49.27</td>
<td>140.25 ± 49.27</td>
</tr>
<tr>
<td>Group IIB</td>
<td>20.50 ± 28.52</td>
<td>44.50 ± 32.18</td>
<td>77.00 ± 36.98</td>
<td>93.50 ± 53.07</td>
<td>100.50 ± 60.07</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>&lt; 0.05</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Significance</td>
<td>HS</td>
<td>HS</td>
<td>S</td>
<td>S</td>
<td>NS</td>
</tr>
</tbody>
</table>

HS – highly significant, S – significant; NS – not significant

### Table 4: Statistical comparison (by unpaired t-test) of *S. mutans* (in cfu/ml) of mean change at various levels between groups I and III

<table>
<thead>
<tr>
<th>Groups</th>
<th>1st day morning</th>
<th>1st day evening</th>
<th>4th day evening</th>
<th>7th day morning</th>
<th>7th day evening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>137.50 ± 53.70</td>
<td>140.25 ± 49.27</td>
<td>140.25 ± 49.27</td>
<td>140.25 ± 49.27</td>
<td>140.25 ± 49.27</td>
</tr>
<tr>
<td>Group III</td>
<td>0.00 ± 0.00</td>
<td>62.50 ± 37.73</td>
<td>95.00 ± 36.89</td>
<td>121.00 ± 43.38</td>
<td>130.00 ± 42.16</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Significance</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>HS</td>
<td>NS</td>
</tr>
</tbody>
</table>

HS – highly significant, NS – not significant

### Table 5: Statistical comparison (by unpaired t-test) of *S. mutans* (in cfu/ml) of mean change at various levels between groups IIA and IIB

<table>
<thead>
<tr>
<th>Groups</th>
<th>1st day morning</th>
<th>1st day evening</th>
<th>4th day evening</th>
<th>7th day morning</th>
<th>7th day evening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group IIA</td>
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<td>28.50 ± 33.25</td>
<td>35.00 ± 37.78</td>
<td>47.00 ± 39.59</td>
</tr>
<tr>
<td>Group IIB</td>
<td>20.50 ± 28.52</td>
<td>44.50 ± 32.18</td>
<td>77.00 ± 36.98</td>
<td>93.50 ± 53.07</td>
<td>100.50 ± 60.07</td>
</tr>
<tr>
<td>p-value</td>
<td>&gt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.01</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Significance</td>
<td>NS</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

NS – not significant, S – significant
S. mutans is a gram positive, facultative anaerobic bacteria commonly found in the human oral cavity and is a significant contributor to tooth decay.

In present study the daily use of chlorhexidine twice, for a week reduces the salivary S. mutans count highly significantly when comparing baseline with all sample levels which has been used for earlier studies.

Sekino S, Ramberg P, Uzel NG, Socransky S, Lindhe J (2003)4 in their study evaluated that daily use of chlorhexidine mouthrinse as an adjunct to careful mechanical tooth cleaning reduces the number of microorganisms that could be detected in saliva sample.

C Rindom Schiott, WW Briner, H Loe (1976)2 evaluated that the number of students with S. mutans present in saliva decreased significantly by treatment with chlorhexidine.

Magnetized water is the water treated with magnetic force, which itself contains magnetism. In other words, when water comprising the properties of minerals is kept in contact with a magnet, then magnetism passes into it and all the water gets magnetized.

Wevangti Vangra (2008)5—water ionization, when water is affected by electromagnetic vibrations, some molecules of water will separate to hydrogen ion (H+) and hydroxyl ion (OH−). Some hydroxyl ions will combine with minerals such as calcium and become calcium bicarbonate which has alkaline property. Magnetized water has pH value about antioxidant property in which, some hydroxyl ions (OH−) combine together and become H2O and oxygen ion (O). This oxygen ion can stop free radical cycle because it is negatively ion 7.6 to 8.5.

When some oxygen ion will combine together and become oxygen, this oxygen can dissolve immediately in that water. If we put the magnetized water in closed bottle, there are small bubbles that get attached to the walls of bottle. It is said ‘Water which has alkaline property, always has oxygen inside’. This gives energy to the cells, prevents development of anaerobic bacteria and stops their growth.

Since magnetized water is alkaline and also as S. mutans is anaerobic bacteria, therefore, its alkaline property stops the anaerobic bacteria to grow, thereby reducing the count.

When comparing the mean change of both groups, the fall in the S. mutans count was highly significant p < 0.001

The present study results demonstrate that group I shows more reduction in S. mutans count than group IIA.

Menendez A, Li F, Michalek SM, Childers NK (2005)6 in their study evaluated that lower concentration of chlorhexidine used in the US (0.12%) may not be sufficiently strong to reduce S. mutans (even in combination with hydrogen peroxide) compared with other concentration, i.e. 0.2%.

Also, the combination of chlorhexidine mouthrinse and hydrogen peroxide did not have a greater effect than chlorhexidine alone in decreasing the oral S. mutans or Streptococci levels.

When comparing the mean change between both the groups on Day 1 (morning and evening) in S. mutans count was highly significant. On day 4 (evening) and day 7, (morning) the fall in S. mutans count was significant p < 0.01 and p < 0.05 respectively and day 7 (evening) the count was not significant p > 0.05.

The results of the present study demonstrate that by day 7 evening the S. mutans count for group IIB was almost in par with group I.

The statistical comparison of mean change of S. mutans at various levels between group I and group III shows that on day 1 (morning and evening), day 4 (evening), day 7 (morning) in S. mutans count was highly significant statistically with p < 0.001.

The day 7 (evening) the fall in count was not significant with p > 0.05.

The present study results show that by day 7 (evening) the S. mutans count was highly significant p < 0.001.

When comparing the mean change at various levels between group IIA and group IIB, the S. mutans count on day 1 (morning) was statistically nonsignificant p > 0.05.

On day 1 (evening) and day 4 (evening), the count was statistically significant with p < 0.05 and p < 0.01 respectively. On day 7 (morning and evening), the S. mutans count was also statistically significant with p < 0.05.

The present study shows that rinsing for 3 minutes with magnetized water has more reduction in salivary S. mutans count than rinsing for 1 minute.
The statistical comparisons of mean change of salivary S. mutans (in cfu) between all the samples of group IIB and group III show that on day 1 (morning) the result points toward the statistically significant difference with p < 0.05. On day 1 (evening), day 4 (evening), day 7 (morning) and day 7 (evening), the fall in S. mutans between both the groups was statistically nonsignificant (p > 0.05).

The study shows that no statistical difference was found in the S. mutans count when water was magnetized for 24 hours and 72 hours. Therefore, magnetizing water for 24 hours also seems to be satisfactory for reducing the S. mutans count but with variable results.

**SUMMARIES AND CONCLUSIONS**

According to the present study:

- When comparing the antibacterial efficacy, Chlorhexidine has shown better reduction in S. mutans count than the magnetized water. Magnetized water has also shown reduction in S. mutans count and therefore, it can be used as an alternative to chlorhexidine.
- The variables—dosage (10 ml) and frequency (twice daily)—are kept constant for all the groups and have significant effect on reducing the S. mutans count and plaque formation. Whereby, these parameters can be kept as standards for rinsing with magnetized water.
- Chlorhexidine (0.2%) has shown more reduction in S. mutans count when rinsing for 1 minute than magnetized water.
- When comparing between 24 hours magnetized water, more reduction in S. mutans count was seen in group rinsing for 3 minutes than 1 minute rinse.
- When comparing between 24 hours magnetized water (3 minutes) and 72 hours magnetized water (3 minutes) equal reduction in S. mutans count was observed which was almost in par with 0.2% chlorhexidine.
- Taste of magnetized water was also well accepted by children.

As already proved, chlorhexidine is the ‘Gold Standard’ for antibacterial and antiplaque effects. Magnetized water has also shown good results for antibacterial effects and, therefore, can be used as an alternative measure to chlorhexidine.

**REFERENCES**