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"Effect of temperature change of 0.2% chlorhexidine rinse on matured human plaque: an in vivo study."

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Abstract:

groups, each group consisting of five subjects that were made to rinse with warm and cold water and chlorhexidine solution at temperature of 47℃ and 18℃ respectively.Plaque The micro-organisms in bacterial plaque comprise a decisive etiological factor in the origin and development of inflammatory periodontal diseases. Plaque is a complex biofilm that contains various microorganisms and forms mainly on teeth and particularly between them, along the gingival margin, and in fissures and pits. This biofilm adheres by a variety of mechanisms for this reason; plaque control plays a significant role in the prevention of gingival and periodontal problems. Both mechanical aids of tooth cleaning and local chemotherapeutics are used for this purpose. 0.2%chlorhexidine (CHX) solution was the first clinically effective mouth rinse that inhibited supragingival plaque formation and thus the development of chronic gingivitis.

Broad-spectrum antibacterial effect against gram-positive as well as gram-negative bacteria, yeasts , dermaphytes, some lipophilic viruses and due to its prolonged substantivity chlorhexidine is still recognized as the ''gold standard'' for chemical plaque control. Various factors such as concentration, time, temperature and pH have an influence on the retention of chlorhexidine in the human oral cavity after rinsing. However, raising the temperature does increase the rate at which chemical reactions take place.[1] Thus the aim of this randomized clinical trial is to evaluate the effect of the temperature change on antibacterial efficacy of Chlorhexidine digluconate.

Aims

The aim of this study is to compare the anti-bacterial efficacy of 0.2% warm and cold chlorhexidine rinse on plaque vitality.

Methods and Material

20 dental students were randomly selected, aged between 18-25 years. The volunteers were instructed to refrain from tooth brushing & not to disturb the plaque growth either mechanically or chemically for three days, so that a matured supragingival plaque was established. The volunteers were divided into four samples were taken 10 minutes before and after rinsing and analyzed under fluorescent microscope.

Results

The percentage of dead bacteria was significantly improved from 93.74% to 73.84%and 95.84% to 28.11%after rinsing with 0.2% cold and warm chlorhexidine solution respectively.

However warm chlorhexidine rinse caused 47.83% greater reduction in percentage of dead bacteria than cold chlorhexidine.

Conclusions

Heated chlorhexidine rinse proved to be a more effective anti-plaque agent than cold chlorhexidine rinse at 0.2% concentration.

Keywords: *Chlorhexidine di-gluconate, anti plaque agents, fluorescence staining*

1.Introduction

Caries and inflammatory periodontal disease are the most prevalent oral diseases, and both result from the activity of dental bacterial plaque. Plaque is a complex biofilm that contains various microorganisms and forms mainly on teeth and particularly between them, along the gingival margin, and in fissures and pits. This biofilm adheres by a variety of mechanisms. If plaque is not removed regularly, the flora evolves and plaque may calcify, forming calculus. For this reason, plaque control plays a significant role in the prevention of gingival and periodontal problems. Both mechanical aids of tooth cleaning and local chemotherapeutics are used for this purpose. 0.2%chlorhexidine (CHX) solution was the first clinically effective mouth rinse that inhibited supragingival plaque formation and thus the development of chronic gingivitis.[1,2,3]

Due to its broad-spectrum antibacterial effect against gram-positive as well as gram-negative bacteria, yeasts ,dermaphytes, some lipophilic viruses and the prolonged substantivity chlorhexidine is still recognized as the ‘‘gold standard’’ for chemical plaque control.[1] Furthermore, alternative antiplaque agents may show the same degree of persistence at the tooth surface as chlorhexidine, but they may not possess the bactericidal and bacteriostatic properties of chlorhexidine. It is this dual antibacterial effect that allows the persistence of the molecule to translate into antiplaque effect, due to the period of time over which bacterial build up and multiplication on the tooth surface is prevented. Chlorhexidine shows different effects at different concentrations; at low concentrations the agent is bacteriostatic, whereas at higher concentrations the agent is rapidly bactericidal.[2,3] Various factors such as concentration, time, temperature and pH have an influence onthe retention of chlorhexidine in the human oral cavity after rinsing. However, raising the temperature does increase the rate at which chemical reactions take place.[4] Thus this study was conducted to evaluate the effect of temperature change of chlorhexidine on its anti-plaque efficacy.

- The aim of this study is to compare the anti-bacterial efficacy of 0.2% warm and cold chlorhexidine rinse on plaque vitality.
  - 20 dental students were randomly selected, aged between 18-25 years.
  - The volunteers were instructed to refrain from tooth brushing & not to disturb the plaque growth either mechanically or chemically for three days, so that a matured supragingival plaque was established.

- The volunteers were divided into four groups:
  - Group I consisting of 5 subjects, they were made to rinse with **cold water** cooled to temperature of 18℃.
  - Group II consisting of 5 subjects, they were made to rinse with **hot** watersolution heated to a temp of 47℃.
  - Group III consisting of 5 subjects, they were made to rinse with **cold** chlorhexidine cooled to a temperature of 18℃.
  - Group IVconsisting of 5 subjects, they were made to rinse with **hot** chlorhexidine solution heated to a temp of 47℃.

2.Methods Of Sample Collection:[1]

- Individual plaque samples were collected from the buccal surfaces of the canine, premolars and first molar with universal curettes.
- Plaque samples were taken both before and 10 minutes after rinsing.
- Samples were then stained with freshly prepared Fluorescein Diacetate (FDA) /Ethidium Bromide (EB) dye and analyzed under a fluorescence microscope , using an excitation filter with a wavelength of 450–490 nm .The preparations were then photographed without delay at 40 X using a no.10 objective . FDA stains living microorganisms green; EB gives a red colorto the nucleic acids of dead bacteria.

3.Analysis Of Data:[1,5,6]

- Data was analyzed using image analysis software (Sigma Scan Pro version 5.0 SPSS Inc, Chicago,USA).
- Red area represented dead bacteria,
- Blue area represented bacteria-free area,
- Green area represented living bacteria.
- Percentage of dead bacteria was calculated before and after rinsing with cold and warm water and chlorhexidine solution.[1]

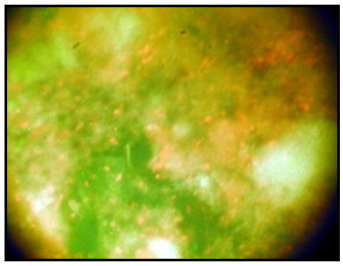

Figure 1: showing living & dead bacteria after rinsing with cold chlorhexidine

Image Recorded After Rinsing With Cold Chlorhexidine

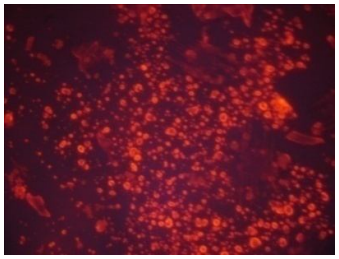

Figure 2: showing living & dead bacteria after rinsing with hot chlorhexidine

Image Recorded Agter Rinsing With Warm Chlorhexidine

4.Statistical Analysis Used

The statistical analysis was done using SPSS version 15 for Windows.The statistical calculations were done with a two-sided paired student *t*-test. Values with $p<0.05$ were accepted as significant.

5.Result

Table 1.shows the % percentage of dead bacteria before and after the rinsing with the cold and warm water respectively.

Subject	Dead before Cold water (%)	Dead after Cold water (%)	Difference (%)	Dead before Warm water (%)	Dead after Warm water (%)	Difference (%)	Difference Warm-Cold water (%)
A	2.7	1.78	-0.92	1.08	0.9	-0.18	0.74
B	3.65	8.59	4.94	5.66	5.26	-0.4	-5.34
C	1.16	1.46	0.3	2.08	2.73	0.65	0.35
D	1.26	1.27	0.01	3.45	3.42	-0.03	-0.04
E	1.06	0.89	-0.17	2.45	2.3	-0.15	0.02
Statistics			*N.S*			N.S	N.S

Table 1

Table 2.shows the percentage of dead bacteria before and after rinsing with 0.2% warm and cold chlorhexidine solution respectively.

The percentage of the dead bacteria prior to the cold rinsing was 6.26% .After rinsing with cold chlorhexidine solution the percentage of the dead bacteria ranges from 22.07 % to 31.84% with a mean value of 19.90%.The percentage of dead bacteria prior to warm rinsing was 4.16%. After rinsing with warm chlorhexidine solution the percentage of dead bacteria ranges from 61.23% to 84.10% with a mean value of 67.73%.There was a greater increase in the percentage of dead bacteria with warm chlorhexidine than cold chlorhexidine. Warm chlorhexidine was 47.83% more effective in killing bacteria than cold chlorhexidine.$p<0.001$ showing it was statistically significant in both the cases.

Subject	Dead before Cold CHX (%)	Dead after Cold CHX (%)	Difference (%)	Dead before Warm CHX (%)	Dead after Warm CHX (%)	Difference (%)	Difference Warm-Cold CHX (%)
A	7.97	25.85	17.88	7.45	67.33	59.89	42.00
B	5.89	23.68	17.79	5.93	63.61	63.61	45.82
C	4.11	31.84	27.73	2.73	69.81	69.81	42.08
D	12.97	27.35	14.37	0.31	84.10	84.10	69.72
E	0.36	22.07	21.71	4.38	61.23	61.23	39.53
X±SD	6.26±1.58	26.16±22.40	19.90±14.81	4.16±1.38	71.89±64.42	67.73±56.82	47.83±35.39
Statistics			$p<0.001$			$p<0.001$	$p<0.001$

Table 2

6.Discussion

Dental plaque represents a part of natural human microbial flora. Their presence in the form of supra and subgingival plaque is strongly associated with plaque-induced diseases. Prevention and control of plaque can possibly be achieved by suppression of oral flora to a extent where colonization does not occur or by direct anti-bacterial action on tooth surfaces.[1,2]The most effective anti-plaque agent, chlorhexidine optimum dose delivered by mouth rinse is generally considered to be 10ml 0.2% of chlorhexidine gluconate.Chlorhexidine has showed considerable plaque inhibition even at a concentration 0.01%.[3]

- 0.2% Chlorhexidine rinse not only prevents formation of new plaque(Loe&Rindom Schiott,1970)but also reduces established plaque[6]
- Concentration of chlorhexidine above 0.2% would almost certainly have produced unacceptable local side-effects in particular taste, taste disturbance and more importantly, mucosal erosion.[8]
- After a single rinse with chlorhexidine, the saliva itself exhibits antibacterial activity for up to 5 hours whereas persistence at the oral surfaces has been shown to suppress salivary bacterial counts for over 12 hours.
- Superior anti plaque effects of Chlorhexidine can be explained in terms of its superior degree of persistence at the tooth surface or, more correctly, its superiorpersistence of antibacterial effect (both bactericidal and bacteriostatic) at the tooth surface. Other agents may show a limited persistence, but their chemical properties mean they are either lost from the tooth surface faster than chlorhexidine or they are bound to the surface in such a way that they cannot interact with a bacterium.[2]
- The aim of this study is to compare the anti-bacterial effect of 0.2% warm and cold chlorhexidine rinse on plaque vitality.
- Various factors such as concentration, time, temperature and pH have an influence on the retention of chlorhexidine in the human oral cavity after rinsing.[4]The temperature of 47^0C was selected since this is the temperature where neither painful sensations nor permanent pulpal damage have been observed at this temperature.

- Following rinsing with water at 47^0Cthere was no change in vitality rates. Thus the combination of heat and chlorhexidine gluconate constitutes the effective parameter.
- To obtain the largest possible amounts of plaque with maximum vitality for quantitative evaluation a plaque maturing period of 72 h was selected for this study. Vitality rates above 90%were found. The microbial vitality corresponds to the portion of vital microorganisms in relation to total bacterial count.
- When plaque quantity is assessed, for instance with indices, this measurement does not give any information about its cellular and acellular content nor concerning the vitality of the cells. Ii is therefore impracticable to analyze the antiplaque effect of a drug when it is not known whether its mechanism of action is dealing with adhesiveness or bactericidal activity orboth.[9]
- The reliability of new staining method as described by Netuschil 1983 helps to estimate the ratio between vital and was dead bacteria present in dental plaque before and after chlorhexidine rinses using this fluorescence test. FDA/EB staining method provides a reliable qualitative test of cell vitality.[5]
- Photographed images give a higher level of accuracy and reproducibility than visual estimation.
- 1 min of rinsing with 0.2% chlorhexidine reduced the vitality rate by 26.16% with cold solution and by 71.89% with warm solution.
- The increase in temperature improved the efficacy of 0.2% chlorhexidine digluconate over the cold chlorhexidine in killing the microorganisms.
- Further clinical investigations will be required to determine to what extent raising the temperature of chlorhexidine mouth rinses reduces development of gingivitis and caries respectively.
- Our results confirm that raising the temperature of anti-plaque agents can increase their efficacy.
- This study concurs with the others studies as it confirms the excellent antimicrobial effect of chlorhexidine, where heated chlorhexidine gluconate proved to be even more effective. Heated mouth rinse solutions can be used instead of cold solutions in dental hygiene in the following situations:

- As a preoperative measure to reduce germ counts, as an intraoperative and postoperative measure and for prophylactic purposes in patients at high risk for periodontitis, etc.

7.Reference

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