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Chlorhexidine Versus Herbal Mouthwash: A Clinical Study

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ABSTRACT

Aim: The aim of this study was to compare the effect of Chlorhexidine mouthwash and Herbal mouthwash against streptococcus mutans on a 8-12 years old school going children.

Materials and Methods: Thirty subjects from a single school were randomly divided into two groups. The salivary count of s. mutans was measured at the beginning of the study and after two weeks, after use of mouthwash. Group A subjects used Chlorhexidine (Rexidiene^{R}) and Group B used Herbal mouthwash (Hiora^{R}). Statistical analysis was done using Wilcoxon signed rank test (non-parametric equivalent to paired't' test) and Kruskal-Wallis Annova. Mean values in each group and were calculated.

Results: A statistically highly significant difference was obtained between each mouthwash at baseline and after use of mouthwash after 2 weeks. However when both the mouthwashes were compared, there was a non-significant difference between mouthwash A and B ($p<.05$) thus showing that Herbal and Chlorhexidine mouthwash was equally effective in reducing streptococcus mutans count

Conclusions: The efficacy of herbal mouthwash was equally effective in streptococcus mutans test therefore can be used as a viable adjunct for maintaining oral hygiene in children.

Keywords: Chlorhexidine, herbal mouthwash, Streptococus mutans .

INTRODUCTION

Recent swing of increasing levels of dental caries is one of the major public health concern in most of the developing countries. Owing to the increased consumption of sugars and reduced exposure to fluoride, children are major risk to it.[1] In India, this trend is steadily increasing both in prevalence and in severity over last five decades.[2] According to the World Health Organization (WHO), 60-90% of students have dental caries.[3]

Streptococcus mutans, one of the prime predisposing factors aids the initiation and progression of dental caries.[4,5] S. mutans in the saliva is associated to the number of colonized surfaces. Wherefore, reducing the concentration of S. mutans in the oral cavity would have a great ease with respect to decreasing the incidence of dental caries.[6] Chemotherapeutic and antimicrobial agents target these factors and reduces their number in oral cavity thus reduces the risk of dental caries.[7] A mouthwash is a medicated liquid which is held in the mouth and swished by the action of perioral musculature to eliminate the oral pathogens.[8]

Chlorhexidine is extensively used antimicrobial agent in dental practice for about three decades.[9] For the reason that it exhibits various side effects such as taste alteration, supragingival calculus formation, and desquamation of oral mucosa, unilateral/bilateral parotid swelling, oral mucosal

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ulceration and parathesia barred its usage.[10,11] In consideration of such side effects the world health organization advice researchers to investigate use of plants and plant extracts.[12] Hiora[R], a herbal mouthwash, contains the advantage of the following ingredients pilu, nagavalli, bibhitaki, yavanisatva, peppermint satva, gandhapura taila, ela. All these ingredients have been proven individually possess high antimicrobial activity against S. mutans.[13]

Hence, this study was planned to compare anti bacterial efficacy of Herbal mouthwash and CHX mouthwashes in school going children.

MATERIALS & METHODOLOGY

The present study was conducted to compare the antibacterial efficacy of herbal mouthwash against chlorhexidine mouthwash on streptococcus mutans counts of school going children aged 8-12 years old in Jodhpur city.

SOURCE OF DATA

Subjects were selected from a private school in Jodhpur city. Microbiological assessment and evaluation was done at Department of Microbiology, Dr S.N Medical College, Jodhpur.

SAMPLE SIZE

A sample of 30 subjects were selected and equally divided into 2 groups.

Group A- Rexidiene[R] mouthwash (0.2% Chlorhexidine)

Group B- Group C- HiOra[R] (Herbal mouthwash)

APPROVAL FROM THE AUTHORITIES:

Permission to conduct the study was obtained from all the concerned authorities

INCLUSION CRITERIA

Subject with at least one active white spot on smooth surfaces (facial or lingual)

Exclusion criteria

- Physical limitations, which precluded the normal tooth brushing and mouth rinsing.
- Marked intra oral soft tissue pathology.
- Medically compromised patients and subjects with history of taking antibiotics 3 months prior to or during the course of study.
- Subjects undergoing orthodontic treatment or with extensive intra prosthesis
- Children who could not brush their teeth or rinse on their own
- Presence of any intraoral soft tissue pathology

Clinical examination was carried out by the chief investigator in the school premises

ASSESSMENT OF DENTAL CARIES

The subject with at least one active white spot on smooth surfaces (facial or lingual) was considered as high caries activity subject. The presence of the active lesions on these surfaces and a high caries activity imply that the subject has a high infection of mutans streptococci which means that he/she belongs to high caries risk group

SALIVA COLLECTION

Stimulated saliva was collected for microbial analysis. The subjects were asked to simulate chewing action with sterilized cotton rolls for 4 min. At the end of 4 min, the students were made to expectorate into sterile container. The stimulated saliva was then transported to the microbiology department within 30 min in ice boxes .The sample were cultured on same day. Saliva sample were again at the end of two weeks after use of mouthwash.

STANDARDISATION OF THE SALIVA COLLECTION TECHNIQUE:

1. The subject did not eat or drink (except water) 1 to 2 hours before collection.
2. The subject did not have any physical exercise before collection.
3. The saliva was collected between 9.30 to 11.30 am.

ADMINISTRATION OF MOUTHWASH

The study was explained to subjects and were instructed the use of mouthwash twice a day (15ml of the mouthwash twice a day each time for 30 seconds) they were requested not to change their routine oral hygiene practices. The children were advised not to eat or rinse for the next 30 min.

INCUBATION AND MICROBIOLOGICAL ENUMERATION

Saliva samples were serially diluted in 6 fold steps in normal saline. The serial dilution was carried out in pre-prepared sterile water blanks of 9ml each. 20 µl of saliva sample were spread on mitis salivarius agar (Hi Media Company, India) supplemented with 0.2 U/ml bacitracin (Hi Media Company, India) and sucrose (15% w/v) using cotton swab for *S. mutans* count. The preparation of media, required sterilization and pouring of plates were done one day before the plating of cultures. All the plates were kept in incubator for one hour before the saliva samples were spread on it. Care was taken that all this procedure of serial dilutions and plating was done in work station called as Laminar Air Flow work station, it provides work space for sterile transfers. The work station used in the present study was vertical flow type. Petri-dishes were then kept in candle jar at 37ºC for 48 hours That is the plates were kept in anaerobic chambers and then placed into incubators at 37ºC for 48 hours. The identification of colonies were done using morphology and characteristics observed under light microscope. The confirmation of MS was done under light microscopy after heat fixed smear slide. Additional confirmation was done using gram staining and catalase negative confirmation test. Microbial counts were further expressed as colony forming units (CFU) per ml of stimulated saliva.

- Score 1 < 10^4CFU/ml.
- Score 2 10^4- 10^5CFU/ml.
- Score 3 10^5 -10^6 CFU/ml.
- Score 4 > 10^6 CFU/ml.

STATISTICAL PROCEDURES

Statistical procedures were carried out in two steps,

- 1. Data compilation and presentation
- 2. Statistical analysis

The data collected was compiled, tabulated, analyzed and subjected to statistical tests. Analysis was done using SPSS software version 17. For intra group comparative statistical analysis of change in microbial counts before and after rinsing was assessed using Wilcoxon signed rank test (non-parametric equivalent to paired't' test) whereas the difference between two groups was assessed using Kruskal-Wallis Annova. Mean values in each group and are calculated.

RESULTS

This clinical study was conducted on 30 school going students.

Table 1 shows the comparison of streptococcus mutans count scores between Group A (Rexidine^R mouthwash) at baseline and after 15days ,it showed statistically significant reduction in Streptococus mutans count (P=0.002) after rinsing with 0.2% chlorhexidine

Table 1: Intragroup comparison of streptococus mutans count score in group A

Group A	n	Mean	SD	p-value
Baseline	15	3	1.07	0.002*
15 Days	15	1.87	0.92	

Table 2 Shows comparison of streptococcus mutans count scores between Group B (Hiora^R mouthwash)at baseline and after 15days ,it showed statistically significant reduction in Streptococus mutans count (P=0.008)

Table 2: Intragroup comparison of streptococus mutans count score in group B

Group B	n	Mean	SD	p value
Baseline	15	2.87	1.06	0.008
15 Days	15	2.43	1.09	

*Wilcoxon matched pairs test

Table 3: Intergroup comparison of Streptococcus Mutans count score.

Group	Baseline		15 days		P value*	
	Mean	SD	Mean	SD		
Group A	3	1.07	1.87	0.92	.7	
Group B	2.87	1.06	2.43	1.09	.17	
*Kruskall Wallis ANOVA						

Table 3 shows comparison between all the two groups at baseline and after 15 days rinsing with mouthwashes ,When both the groups were compared with **Kruskall Wallis ANOVA** they showed that there is statistical no difference in Mutans streptococci count before and after rinsing with mouthwashes.

DISCUSSION

Currently, use of mouthwashes is increasing to prevent dental caries. The number of mouthwash variants in the world has grown from 15 in 1970 to nearly about 113 in 2012.[14] With the increasing number of mouthwashes our goal should be to provide a mouthwash with maximum benefits and fewer side effects to children. Thus the present study was undertaken to evaluate the efficacy of herbal mouthwash and a chlorhexidine containing mouthwash on salivary Streptococcus mutans on school children aged 8-12 years.

In the present study saliva samples were used in this study to assess the microbial aspect of dental caries. According to Larmas and Messer, salivary mutans streptococcal tests can be used for the pre selection of patients for dental examination, display of cariogenic infection and evaluation of the effectiveness of chemotherapeutic rinses and providing for an objective measure of treatment outcomes. [15,16]

Chlorhexidine is a broad-spectrum biocide effective against array of Gram-positive bacteria, Gram-negative bacteria and fungi. It inactivates microorganisms with a broader spectrum than other antimicrobials. It has both bacteriostatic and bactericidal action, depending on its concentration. In vitro, chlorhexidine can kill nearly 100% of Gram-positive and Gram-negative bacteria within 30 seconds. Since chlorhexidine formulations can destroy the majority of categories of microbes, there is limited risk for the development of opportunistic infections.[17] Chlorhexidine is a positively-charged molecule that binds to the negatively-charged sites on the cell wall; it destabilizes the cell wall and interferes with osmosis. The bacterial uptake of the chlorhexidine is very rapid, typically working within 20 seconds. In low concentrations it affects the integrity of the cell wall. Once the cell wall is damaged, chlorhexidine then crosses into the cell itself and attacks the cytoplasmic membrane (inner membrane). Damage to the cytoplasm's delicate semipermeable membrane allows for leakage of components leading to cell death. In high concentrations, chlorhexidine causes the cytoplasm to congeal or solidify.[18] Despite such potent antimicrobial agent, downside of chlorhexidine containing mouthwashes are presence of alcohol.

Mouthwashes contain pharmaceutical-grade denatured alcohol as a vehicle to deliver antimicrobial ingredients. The alcohol serves the purposes of solubility, preservability and germicidal activity.[19] Mouthwashes containing alcohol should not be prescribed for children. Its major side effects are Xerostomia, burning or sore sensation, oral cancer, presence of oral pain, burning sensation, difficulty to use in patients with oral sensitivity and risk of accidental alcohol ingestion in children.[20] Ingestion of large amount of alcohol in mouthwash affect normal glycogenolysis and glyconeogenesis, causing hypoglycemia.[21] So it is our paramount responsibility to search for alcohol free mouthwashes for children.

Herbal mouthwash contains a natural ingredients called phytochemical that possesses anti-microbial and anti-inflammatory effect. They function without alcohol, artificial preservatives, flavor, or colors. Most herbal mouthwashes are safe alternative to pregnant women, people with dry mouth, diabetic and to children. In place of artificial colors it

contains vegetable juices such as beetroot, tomato, carrot, and annatto to add color.[22]

In the present study both the group showed statistically significant difference in streptococcus counts pre and post rinsing. When inter group comparison were done, they showed no statistical significant difference between the groups post rinsing (p=0.17). Consequently the present study shows that both the mouthwash used were effective in reducing the streptococcus mutans count.

In a review of literature of studies contrast results were been found Deshmukh M A et al when compared efficacy of CHX, Hiora[R] regular and probiotic mouthwashes on plaque accumulation, gingival health and oral hygiene status found no statically difference in three of them.[23] Narayan A and Mendon C compared Hiora[R] and CHX mouthwashes were shown to have equal anti-plaque efficacy [24] whereas Singh A et al., where statistically significant difference in plaque parameters was observed with CHX compared to Hiora[R] Regular. [25]. Biswas G et al., concluded that chlorhexidine is better option than herbal mouthwash in reducing gingival and plaque index score.[26]

Since, there was no significant difference between the two mouthwashes, with regard to their efficacy in reducing *S.* mutans, the use of a herbal based mouth wash appears to be a suitable choice for regular use in children.

CONCLUSION

It can be concluded that the mouthwashes, CHX and Herbal in reducing streptoccus mutans count. Authors suggest the promotion of Herbal mouthwash in alternate to Chlorhexidine. However long term clinical studies are required.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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