An *In vivo* Comparison of The Bacteriostatic Efficacy of Two Commercially Available Mouthwashes Viz. Triclosan And Fluoride Based Mouthwashes Using A Simple Chair Side Caries Activity Test - Oratest In Children

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Abstract

Aim: To compare the bacteriostatic efficacy of two commercially available mouthwashes viz. triclosan and fluoride based mouthwashes using a simple chair side caries activity test - Oratest in children. Materials and methods: Sixty children of the age groups 6-12 years were selected for the study (30 children – caries free, 30 children - DMFT score \geq 3) and were equally divided into two groups that is Group I (Kidodent group) and Group II (Amflor group). These groups were further subdivided into Group I A (15) caries free children and Group I B (15) children with DMFT score \geq 3 Group II A (15) caries free children and Group I B (15) children or Kidodent and evaluated using Oratest for caries activity. Results: The data obtained was tabulated and compared using students paired t test. In the Kidodent group, the time taken for the oratest in both the subgroups I A and I B was found to be significant pre and post rinse. (p<0.001). Similar results were also found in the Amflor group for both subgroups II A and II B, following pre and post rinse. (p<0.001). On comparing the efficacy of Kidodent and Amflor pre and post rinse there was no statistical significant difference between the groups. Conclusion: Although we found no significant difference between both the mouth rinses, with regard to their efficacy in reducing *S. mutans*, the use of a low fluoride–xylitol based mouth rinse can be recommended for regular use in children.

Key Words: Oratest, Mutans strepococci, Mouth washes

Introduction

Recent concept of dental caries is considered as an interaction between genetic and environmental factors where in social, biological, psychological and behavioral factors are expressed in a highly complex interactive manner [1]. But the indispensable part in the comprehending the caries process is that it does not occur in the absence of dental plaque which consists mainly of bacteria or dietary fermentable carbohydrate, thus considering it a dieto-bacterial disease [2]. Mutans *Streptococci* are the main etiological microorganisms in dental caries [3].

The growing interest in the microbiological aspects of dental caries has led to the invention of a variety of diagnostic procedures. A number of caries activity tests have been developed to detect and diagnose the presence of conditions associated with increased caries risk and have been widely used in, monitoring and motivation of patients with dental caries [4].

One such chair side simple, economical, non-invasive method used for the detection and diagnose oral microbial level based on the rate of oxygen depletion in expectorated milk sample, called oratest, was developed by Rosernberg et al [5] in 1989. Indicator dye methylene blue was used in this test. The caries activity of an individual is assessed by evaluating the time taken for the indicator dye methylene blue in the expectorated milk after rinsing of the mouth to change the color, and this is inversely proportional to the number of organisms in the expectorate. Initially studies were done on oratest for gingivitis, halitosis and also as a method to monitor denture hygiene but later in 1996 Patalay et al attempted to use oratest as a method to determine caries activity since dental caries has been currently accepted to be a specific microbial disease [6].

A large body of evidence supports the opinion that microorganisms present in dental plaque constitute the primary etiological factor in dental caries, gingival and periodontal disease [7-9]. Daily oral hygiene aims at controlling plaque formation, which is a key factor in preventing dental caries and periodontal disease [10]. The most common and undisputed means of performing oral hygiene is the use of a toothbrush and fluoridated dentifrices. However, daily oral hygiene also depends on the dexterity of individuals [11]. In children, factors like lack of dexterity and individual motivation and monitoring limit the effectiveness particularly at interproximal sites which necessitates the use of chemotherapeutic agents like mouth washes for control of plaque [12].

Mouth rinses have generally been found to be efficacious means of controlling the incidence of dental caries. Hence the study was carried to compare the efficiency of two commercially available mouthwashes using a simple chair side caries activity test : Oratest as a measure of oral microbial levels.

Principle

The rate of oxygen depletion by the microorganisms forms the bases for Oratest. Indicator dye methylene blue is used in this test. Under an aerobic environment the bacterial enzyme; aerobic dehydrogenase transfers the electrons or protons to oxygen. As the aerobic organisms utilize the oxygen an anaerobic environment is attained, methylene blue [redox indicator] acts by accepting an electron and gets reduced to leukomethylene blue. The reduction of methylene blue to

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leukomethylene blue reflects the metabolic activity of the aerobic microorganism [13]. Sterile milk is used to rinse the mouth as it aids in dislodging the micro-organisms and it also yields a substrate for their further metabolism. The formation of leucomthylene blue can be easily perceived because of the white color of milk.

Material and Methods

All the children were asked to rinse their mouth for a time period of 30 seconds vigorously using 10 ml of ultra-high temperature sterilized cow's milk and the expectorate was collected in a sterile beaker. 3 ml of the expectorate was immediately transferred with a disposable syringe to a screw cap test tube which contained 0.12 ml of 0.1% methylene blue. The contents of the test tube were thoroughly mixed and were placed on a stand in a well-illuminated area. The test tube was observed every 5 min to detect any color change (blue to white) using a magnifying mirror. The time taken for the initiation of the color change within a 6 mm ring was recorded.

Post one hour since oratest the children in Group I were asked to rinse their mouth using 10ml Kidodent Group II were asked to rinse their mouth using 10 ml Amflor for 30 secs. Both mouth rinses were alcohol free. After rinsing, the children were advised not to eat or drink for 30 min. Oratest procedure was repeated in both the groups and the time taken for the initiation of the change in color noted. The results were tabulated and analyzed using students paired t test.

Results

In the kidodent group, the time taken for the oratest in both the subgroups IA and IB was found to be significant pre and post rinse. (p<0.001) (*Table 1*).

Table 1. Comparative evaluation of the time taken for oratest pre and post rinse in the kidodent sub- group.

	Sub- groups	N	Mean time taken	Std deviation	P value
	lb	15	20.67	3.848	<0.001
Pre-rinse	la	12	32.67	4.438	
	lb	15	38.13	6.49	<0.001
Post rinse	la	12	57.75	8.125	

Table 2. comparative evaluation of the time taken for oratest pre and post rinse in the amflor sub- group.

	Sub groups	N	Mean time taken	Std deviation	P value
	llb	15	18.73	2.789	<0.001
Pre-rinse	lla	12	31.83	3.186	
	llb	15	36.5	4.406	<0.001
Post rinse	lla	13	58.38	9.465	

Similar results were also found in the amflor group for both subgroups IIA and IIB, following pre and post rinse. (p<0.001) (*Table 2*).On comparing the efficacy of kidodent

and amflor pre and post rinse for the subgroups IA and IIA, subgroups IB and IIB, was found to be statistically non-significant (*Figure 1* and *Figure 2*).

Discussion

Many children have unsatisfactory oral and general health because of active and uncurbed dental caries. Its significance in over all human health has minimized owing to its non-life threatening nature and ubiquitousness. Initiation of dental caries and the microbial composition of plaque have predominantly involved either S mutans or Lactobacilli. As Children with high dmft have increased *S mutans* count, a variety of anti-plaque agents has been developed and assessed for their ability to control *S mutans* [2].



Figure 1. Comparison of effectiveness of Kidodent and Amflor mouthwash pre and post rinse in the sub groups Ia and Ib.





As plaque is a complex aggregation of various bacterial species, no single agent can be effective in complete elimination of plaque. An approach to increase the efficacy of anti-plaque agent and to reduce the adverse effects may be to combine two or more agents. Although, chlorhexidine is the gold standard among the mouth rinses that are available, yet due to its disadvantages many new mouthwashes either having single active agents or combination agents have been developed to be used in children.

Triclosan is used as an antimicrobial agent in many oral hygiene products due to its broad-spectrum antimicrobial activity and is found to be effective against S. mutans at low concentration [14]. It acts by adsorbing to the lipid portion of the bacterial cell membrane and in low concentrations interferes with vital transport mechanism. Since triclosan alone is found to be less effective it is usually combined with other agents like fluoride, xylitol to increase the efficacy against oral microflora [15].

The effects of fluoride on bacterial metabolism are also well known. Fluorides inhibit several essential enzymes in oral bacteria [16]. The current evidence indicates that fluoride has a multitude of direct and indirect effects on the bacterial cell, which have a significant influence on those organisms in dental plaque and the low potency-high frequency rinsing may be more beneficial [17] amine fluoride has been investigated as an active ingredient in the mouthwashes. The long chain AmF molecule has surfactant properties, which reduces the bacterial adherence to the enamel surface, thus leading to decrease in the plaque growth [18,19]. Xylitol is non-sugar sweetener permitted for use in food products. Passive effects of xylitol is that it is non-fermentable and non-cariogenic, whereas active caries prevention effects as bacteriostatic and cariostatic [20-23].

In our study the efficacy of two mouth rinses Kidodent mouth wash (xylitol (5%), sodium fluoride (0.05%) and triclosan (0.03%) fluoride 228 ppm) (Group I) and Amflor mouth wash (Amine Fluoride, Proylene Glycol fluoride conc (480 ppm)) (Group II) to reduce *S mutans* was assessed using oratest. In Group I the time taken was 20.67min and 38.13 min in caries active children and in Group II the mean time taken was 18.73 min before rinsing and 36.53 min after rinsing in caries active children .The results were in accordance with the study conducted by Patalay et al. and Anand et al [6,24].

In routine clinical practice, we face several situations where oral hygiene maintenance has to be monitored. Such situations include cases with dental caries, gingival and periodontal disease and appliance therapy. Oratest could prove useful in all the situations mentioned above, it can be easily learnt by the auxiliary personal and hence can be a used as a diagnostic tool in school health programs [21].

Dental caries activity tests have been widely used in the assessment, monitoring and motivation of patients with dental caries and still caries activity test is under the continuous challenge due to its multi factorial nature [22]. Investigators have suggested relationships between the presence streptococcus mutans and evidence of dental caries and others proposed to detect microbial products to measure the caries activity. Unfortunately, many of these approaches require extensive work up time and additional equipment, including incubators and microscopes for morphological count, often samples must be sent out for analysis. A simple in expensive technique that does not demand sophisticated skills or valuable costly "chair side" time, required in order to expedite the diagnosis and appropriate management of dental caries. Further the positive results can easily visualized by the practitioner, child and the parent and thus can be used to motivate [24].

In the present study, when all 60 subjects were subjected to two different mouth rinses, significant co relation was found between time required for color change in either of the groups suggesting considerable reduction in microbial counts. Tal Haim and Rosenberg Mel observed similar results when they compared oratest scores with commonly used techniques for clinical evaluation of plaque levels and gingival inflammation [5]. Oratest can be used as a diagnostic tool to assess the caries activity since a definite relationship exist clinically with caries status and microbiologically with the *streptococcus mutans* count of the individual. We believe that this method will be useful, both at the individual level and community level, for example, when evaluating important caries risk factors in a single case or monitoring a patient undergoing preventive treatment in a given population. Oratest can serve as one measure, in an attempt to identify groups of persons with an increased risk to develop caries.

Dental health education has been considered to be an important and integral part of dental health services and has been delivered to individuals and groups in settings such as dental practice, schools, work place and day care centers. The goals of the interventions have also been broad, so that knowledge, attitudes, intentions, beliefs, behaviors, use of dental services and oral health status have all been targeted for the change [23]. In private practice, the clinical effectiveness of fluoride mouth rinsing assumes paramount importance. However, the value of fluoride mouth rinsing as a public health measure depends not only on its effectiveness, but also on its costs of implementation. Cost effectiveness determination should be made for such rinsing regimen under consideration, and the procedure which is the most cost effective results in the largest total number of surfaces protected in a population, should be adopted [25,26].

Conclusion

Although we found no significant difference between both the mouth rinses, with regard to their efficacy in reducing *S. mutans*, the use of a low fluoride–xylitol based mouth rinse can be recommended for regular use in children. Oratest is a potential whole mouth diagnostic test to measure microbial load in intraoral diseases of epidemiological importance like periodontal disease and caries. Oratest can be used as a good educational and motivational tool for patients school and community dental health program. It also serves as an index for the success of therapeutic measures such as mouth rinsing employed in routine personalized preventive measures.

Further scope lies in establishing the sensitivity of this technique, improving specificity and establishing a grading system for this method so that results obtained using this technique may be universally standardized.

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