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Research Article

comparative evaluation of electrochemically activated water and 0.2%chlorhexidine as a mouth rinse on salivary streptococcus mutans levels in children- An environmental friendly alternative

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ABSTRACT

Context: Due to the resistance of bacteria to most of the commonly used biocides, there is a need to explore new methods of disinfection to help maintain effective bio burden control, especially within the healthcare environment. Electrochemically activated solutions (ECAS) may represent a viable alternative to commonly used disinfectant solutions and a multitude of systems for the generation of active killing solutions.

Aim: To evaluate and compare the antibacterial efficacy of conventionally prepared electrochemically activated water and commercially available 0.2% chlorhexidine as a mouth rinse on salivary Streptococcus mutans levels in children.

Materials and methods: A total of 120 children between the age group of 5 to 12 years with deft ≥5 were selected. The baselines samples were collected before starting with the rinses. They were then divided into three groups. Group I (Control), Group II (0.2% chlorhexidine) and Group III (Electrochemically activated water). Both the groups II and III practiced rinsing with respective mouth wash for 1 min for 15days once in a day. The samples were collected at 1 day, 7 days and 15 days and sent to microbiological laboratory for S.mutans count.

Statistical analysis used: One way ANOVA test with Tukey post hoc analysis

Results: After 15 days, there was statistically significant difference in the mean values of S.mutans count between three groups. Post hoc analysis showed that mean values of S.mutans count were statistically significantly higher for Group I than Group II and Group III.

Conclusion: Electrochemically activated water showed statistically significant reduction in S.mutans count and was equally effective as 0.2% chlorhexidine .

Keywords: S.mutans, Colony forming units, 0.2% Chlorhexidine, Electrochemically activated water

INTRODUCTION

Modern concepts consider dental caries as an interaction between genetic and environmental factors in which social, behavioural, psychological, and biological factors are expressed in a highly complex interactive manner. But the important part in the understanding of the caries process is that it does not occur in the absence of dental plaque or dietary fermentable carbohydrate hence, is considered a dieto-bacterial disease.[1]

Streptococcus mutansis a gram positive, facultative, anaerobic bacteria commonly found in the human oral cavity and is a significant contributor to tooth decay.[2] The role played by bacteria in initiation of dental caries and periodontal diseases is well established. The removal of plaque is utmost important to control

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dental caries that is commonly maintained by mechanical methods. In children, factors like lack of dexterity and individual motivation and monitoring limit the effectiveness of tooth brushing. They also experience difficulty in maintaining adequate plaque control, particularly at interproximal sites¸ which necessitates the use of chemical agents for control of plaque.[1]

Among the chemotherapeutic agents used in mouth washes, chlorhexidine is the "gold-standard" or positive control for comparison with other substances due to its proven efficiency.[1] It is a broad-spectrum antimicrobial agent with effect on gram positive and gram-negative bacteria as well as on fungi and some viruses.[3] The most persistent reduction of Streptococcus mutans has been achieved by chlorhexidine mouthwashes.[4]

Due to the resistance of bacteria to most of the commonly used biocides, there is a need to explore new methods of disinfection to help maintain effective bio burden control, especially within the healthcare environment.[5] Electrochemically activated solutions (ECAS) may represent a viable alternative to commonly used disinfectant solutions and a multitude of systems for the generation of active killing solutions.[6] It has been shown to have broad-spectrum antimicrobial activity, and have the potential to be widely adopted within the healthcare environment due to low-cost raw material requirements, ease of production, rapid disinfection time, requires little operator skill, limited toxicity, environmentally compatible and evidence of being anti-inflammatory.[5]

Till date, there are no studies evaluating the use of electrochemically activated water as a mouth rinse in children. Taking this into consideration, the purpose of this study is to evaluate and compare the antibacterial efficacy of conventionally prepared electrochemically activated water and 0.2% commercially available chlorhexidine as a mouth rinse on salivary Streptococcus mutans levels in children.

Subjects and Methods:

The present study was conducted in the Department of Pedodontics and Preventive Dentistry, Mamata Dental College and Hospital, in association with Department of Microbiology, Mamata Medical College, Khammam. The study was approved by Ethical review committee and Institutional review board.

Inclusion 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 ECA water as oral rinse earlier
- No history of oral prophylaxis done for at least 3 months prior to study.

Exclusion criteria:

- Children who were physically and medically handicapped
- Children who were on medications (antipyretic drugs, bronchodilators, multivitamin syrups)
- Children with intra oral soft tissue pathology
- Children undergoing orthodontic treatment
- Children with extensive intra oral prosthesis

A randomized controlled clinical study was carried out in 120 school going children who met with the inclusion and exclusion criteria from primary schools of Khammam between the age group of 5 to 12 years with deft $\geq$5 for a period of 2 weeks. They were then divided randomly into 3 groups :

- **Group I**: Control (n=40)
- **Group II**: 0.2% Chlorhexidine mouth rinse(Colgate Palmolive, USA) (n=40)
- **Group III**: ECA water mouthrinse (GT Solutions, Chennai, India.) (n=40)

The data was collected on a self-designed proforma which records the demographic data and deft index. All the subjects included in the study were examined by a single qualified examiner using ADA (American Dental Association) Type III specification. The deft recording was done according to the criteria for the dentition status assessment outlined in basic oral health surveys 1997.

The salivary sample collection method was described by Nayak et al (2012).[7] Baseline saliva samples were collected before starting with the rinses. The subjects were informed not to eat or drink anything (except water) 2 hours prior to saliva collection. For this purpose unstimulated

whole saliva was collected between 9.30-11.30 am during the school hours to match the circadian rhythm. Care was also taken to avoid saliva collection before heavy physical exercise. Children were told to swallow the pre-existing saliva in order to clear mouth of any residual unstimulated saliva. After this, each student was asked to collect saliva in the mouth for a minute 1 ml of unstimulated saliva was collected by spitting method in a previously labeled sample collecting vial containing 2ml of transport media (Thioglycollate media, Himedia, Mumbai) and transported in a transportation box to the microbiological laboratory, Mamata Medical College, Khammam where it was processed immediately **.**After collection of baseline samples, the subjects were given respective mouth rinse (10ml) as per the groups and were asked to rinse as instructed, under supervision daily for 1 minute.

Saliva samples were collected from the groups ie .,Group II and Group III after 1 day, after 7 days and after 15 days following the same method as for baseline saliva sample collection. The salivary samples were vortexed, to uniformly mix the saliva and the transport media using a cyclomixer. The culture media used was Mutans sanguis agar (Himedia, Mumbai).Using an inoculation loop (4 mm inner diameter) 10 µL of the vortexed 1:5 dilution sample was streaked in duplicate on Mutans sanguis agar plates (selective for Streptococcus mutans). The agar plates were incubated anaerobically for 48 hours at 37°C in 5% carbon monoxide in nitrogen by placing in 10 litre anaerobic jar (YORCO, York Scientific Industries, Chennai). 1.5 litre anaerobic gas pack (Himedia, Mumbai) and indicator tablet (Himedia, Mumbai) were placed in anaerobic jar to maintain anaerobic environment. Following incubation, counts were made of colonies with morphological characteristic for Streptococcus mutans on the Mutans sanguis agar. Streptococcus mutans formed rough, heaped, irregular colonies resembling frosted glass. Mostly crumbly which were white, gray or yellow in colour and 0.5 and 2 mm in diameter**.** Colony counting was done with colony counter (YORCO, York Scientific Industries, Chennai) under magnifying glass and the count of Streptococcus mutans was expressed as the number of colony forming units per millilitre (CFU/ml) of saliva. Semiquantitation of the number of colonies was done by multiplying the actual colony count with $1x10^3$ because of the part that the saliva sample was diluted one thousand times (1:5 dilution). The obtained values of all the groups were subjected to statistical analysis.

Results:

The results obtained were subjected to statistical analysis. The mean values, standard deviation for the groups were analysed using the Statistical Package for the Social Sciences software, version 24.0. Intra group comparison was made using 't' test. One way ANOVA test with Tukey post hoc analysis was used for inter group comparison. P value of <0.05 was considered as statistically significant.

The mean scores for CFU were for Group I at baseline, 1 day, 7 days and 15 days the mean values are 78.95±22.396, 78.73±17.729, 78.60 ±19.903 and 76.90±20.724 respectively. For Group II at baseline, 1 day, 7 days and 15 days the mean values are 91.30±19.032, 76.53±18.903, 59.95±18.490 and 44.08±15.844 respectively. There was significant reduction in the mean values of Streptococcus mutans count at the end of 15 days. For Group III at baseline, 1 day, 7 days and 15 days the mean values are 86.80±23.833, 70.20±20.705, 54.88±17.601 and 42.68±11.889 respectively. There was statistically significant reduction in the mean values of Streptococcus mutans count.(Table 1)

Table 2, Graph 1 shows that at baseline, there was no significant difference in the mean values between the three groups. Post hoc analysis showed that there was no significant difference between the three groups. After 1 day, there was statistically significant difference in the mean values of S.mutans count between the three groups. Post hoc analysis showed that there was no significant difference between Group I and Group II but mean values of S.mutans count of Group I and Group II were significantly higher than Group III. After 7 days, there was statistically significant difference in the mean values of S.mutans count between the three groups. Post hoc analysis showed that mean values of S.mutans count were statistically significantly higher for Group I than Group II and Group III. After 15 days, there was statistically significant difference in the mean values of S.mutans count between three

groups. Post hoc analysis showed that mean values of S.mutans count were statistically significantly higher for Group I than Group II and Group III.

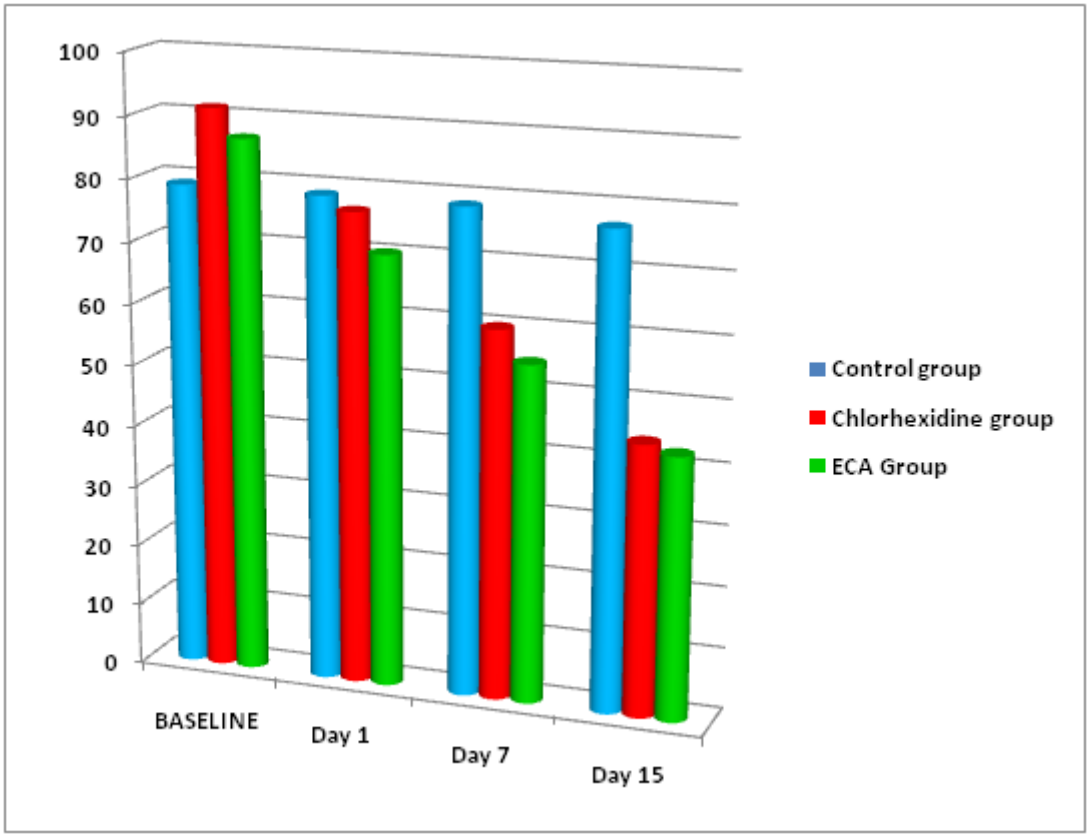
Table 1: Intra group comparison of three groups with 't' test

Group	N	Days of collection	Mean	Std. Deviation	Std. Error
I	40	Baseline	78.95	22.396	3.541
		1Day	78.73	17.729	2.803
		7Days	78.60	19.903	3.147
		15 Days	76.90	20.724	3.277
II	40	Baseline	91.30	19.032	3.009
		1 Day	76.53	18.903	2.989
		7 Days	59.95	18.490	2.924
		15 Days	44.08	15.844	2.505
III	40	Baseline	86.80	23.833	3.768
		1 Day	70.20	20.705	3.274
		7 Days	54.88	17.601	2.783
		15 Days	42.68	11.889	1.880

Table 2: Inter group comparison of three groups with one way ANOVA and Tukey post hoc analysis

	Group	N	Mean	Std. Deviation	Std. Error	P Value	Post hoc
Baseline	1	40	78.95	22.396	3.541	0.06 No significant difference	No significant difference
	2	40	91.30	19.032	3.009		
	3	40	86.80	23.833	3.768		
	Total	120	85.68	22.260	2.032		
1Day	1	40	78.73	17.729	2.803	0.038[a]	1=2>3
	2	40	76.53	18.903	2.989		
	3	40	70.20	20.705	3.274		
	Total	120	74.48	19.528	1.783		
7Days	1	40	78.60	19.903	3.147	0.001[b]	1>2=3
	2	40	59.95	18.490	2.924		
	3	40	54.88	17.601	2.783		
	Total	120	62.81	22.050	2.013		
15 Days	1	40	76.90	20.724	3.277	0.001[b]	1>2=3
	2	40	44.08	15.844	2.505		
	3	40	42.68	11.889	1.880		
	Total	120	49.25	26.554	2.424		

a- Significant; b- highly significant



Graph 1: Inter group comparison of three groups with one way ANOVA and Tukey post hoc analysis

Discussion:

Dental caries is a chronic contagious disease caused by several interacting factors, which results in the irreversible destruction of the mineralized structures of teeth, compromising their vitality and fixation in the maxillo-mandibular complex.[8]

Human oral cavity is an incubator, which provides nutrition, shelter and facilitates growth of numerous microorganisms.[9] The surface is constantly colonized by microorganisms to form complex biofilm. This process is partially controlled by quorum sensing, an inter bacterial communication mechanism that is dependent on population density and is associated with radical changes in protein expression pattern. Amongst them, Mutans streptococci have been regarded as one of the main culprit microorganisms of oral cavity and initiates dental caries.[9] It can produce large amounts of extracellular glucan from sucrose by the enzyme glucosyl transferase. The production of glucan and large amounts of lactic acid by fermentation of carbohydrates constitutes major virulence factors in the causation of dental caries which is proved beyond doubts.[10]

In India, children comprise 40% of a rapidly growing population. The prevalence of dental caries varies from 33.7%-90% and is increasing at an alarming rate.[10] There are ample of evidences from both cross sectional and longitudinal studies showing the strong association between S. mutans and dental caries. All the available evidence indicates that any preventive strategy should have S. mutans as its principal target.[11] In the present study children of age group 5-12 were selected, as they are school going children and they represent end point for caries history of their deciduous teeth.[12]

Saliva secretion varies during the day and is influenced by temperature, season, hydration status, actual health condition (including medication intake) and mood. Nevertheless, the value of saliva as a diagnostic specimen is being increasingly recognized. Therefore, despite its limitations, saliva collection will become a growingly common diagnostic procedure, which will require standardization and accuracy. [12]

In the present study, proposal for standardized collection of whole and glandular saliva: Collect saliva samples always at the same time of the day, preferably between 9:00 and 11:00 a.m, refrain from eating and drinking at least 90 min before collection. If applicable, stop the use of drugs that might affect salivary secretion for at least 1 day or longer (when the drugs have a long half-life). [12]

Jenkins (1978) stated that resting saliva rather than stimulated saliva should be used in the analysis of correlations between salivary factors and oral health in individuals.[13] In the present study unstimulated salivawas used to assess the parameters, as it has been reported that the unstimulated saliva represents the basal salivary flow rate.[7]

Dawes (1987) reported several different ways of collecting unstimulated whole saliva. They are draining method, spitting method, suction method and swab method. It is better to collect the unstimulated saliva samples using the spitting method, because the stimulation of saliva secretion is more slight in the spitting method than in the suction method and spitting method appeared to be the most reproducible and convenient.[14] In the present study to avoid contamination, unstimulated saliva was collected by spitting method into the sterile plastic vials in the morning 9.30-11.30 am to match the circadian rhythm. Thioglycollate transport media was used as a transport media so that the vitality of the organisms is maintained which was in accordance with study done by Nayak et al (2012).[7]

Children with high dmft have increased S.mutans count.[1] So in the present study children with high caries levels, deft $\geq$ 5 were selected and microbiological and clinical correlation was done by assessing *S. mutans* count with dental caries.

Detection and quantitation of microbes is necessary to understand the disease process. Mouth mirrors and probes provide assistance in diagnosis of dental caries, caries activity, but the exact quantification cannot be done without microbiological procedures. So conventional culture has been employed since long for detection and quantitation of microbes and if the strict adherence to procedures is followed, such as sterile lab conditions, strict anaerobic/aerobic protocols maintained produces predictive results.[15]

Salivary levels of *S. mutans* are directly related to the number of tooth sites colonized and to their proportion in dental plaque. As a result variety of antiplaque agents has been examined for their ability to control S.mutans.[16]Plaque control is the primary level of prevention of caries and periodontal disease. Effective plaque control measures will bring about change in quantity and composition of plaque biofilm. This can be achieved by mechanical or chemical plaque control measures.[17]

Mechanical plaque control is the most dependable oral hygiene measure, but mechanical oral hygiene methods of plaque removal require time, motivation and manual dexterity. In children, factors like lack of dexterity, individual motivation and monitoring, limit the effectiveness of tooth brushing.[1] Mechanical control of dental biofilm has a somewhat limited success. Thus, it seems reasonable to control caries by agents that either prevent the formation of or disrupt biofilms of teeth or inhibit acid formation or stimulate base formation by dental biofilms.[11]

In the present study the children were told to rinse using the mouthrinses for 60 seconds which was in accordance with study done by Nayak et al (2012)[7]in which they determined the maximum time up to which children could rinse without any discomfort was 60 seconds.

Chlorhexidine gluconate, a cationic bis-biguanide was introduced for human use in 1957 in Great Britainand its plaque inhibition was first investigated by Schroeder in 1969.[2,18] Chlorhexidine (0.2%) mouth rinse is considered as a 'Gold Standard,' which is commercially available and effective mouth rinse to inhibit supragingival plaque formation. Rindom C, Briner WW, Loe H (1976) found a reduction of 30 to 50% in the population of *S. mutans* after rinsing with 10 ml of 0.2% chlorhexidine mouth rinse once daily.[2]

Woodcock presented the first review on chlorhexidine. After single rinse with chlorhexidine, saliva itself exhibits antibacterial activity for about 5 hours and suppresses salivary bacterial counts for over 12 hours. Periodically repeating several rinses of chlorhexidine, reduced the number of aerobic and anaerobic species by 80

to 90%.[15] In the present study after single use of chlorhexidine mouth wash for one day showed better reduction of salivary Streptococcus mutans levels than control after 1 day which were in accordance with study done by Parkar SM, Thakkar P, Shah K (2013)[11]who concluded that chlorhexidine mouthwash was most effective in reduction of bacterial count from baseline to 1 day.

In present study the daily use of chlorhexidine, for 7 days reduces the salivary *S. mutans* count highly significantly when comparing with baseline salivary sample levels which was in accordance with study done by Sekino et al (2003)[19], Sari E, Birinci I (2007).[20]

In this present study, the children who used 0.2% chlorhexidine mouthwash showed significant reduction in number of colony of S. mutans at the end of 15 days. These results were in par with study done by Kulkarni VV, Damle S G (2003) who concluded that chlorhexidine mouth rinses are more efficient in reducing Mutans streptococci count in saliva as compared to other mouth rinses.[9]

Chlorhexidine mouthwash cannot have long-term effects on decreasing oral bacteria and *S. mutans* increases again after weeks or months. On the other hand chlorhexidine has minor anti-caries effect.[21]

In oral use as a mouth rinse, chlorhexidine has been reported to have a number of local side effects. These side effects are brown discoloration of teeth, some restorative materials, the dorsum of tongue, taste alteration, oral mucosa erosion, and enhanced supragingival calculus formation. Chlorhexidine also has a bitter taste which is difficult to mask completely.[15]

Despite the proven antimicrobial activity of chlorhexidine, studies have shown that microorganisms contained in a biofilm structure become resistant to this antimicrobial disinfectant.[22,23]This advocates the need for supplementary study and new antibacterial agents that are strongly against Streptococcus mutans, with marginal consequences on the oral tissues, especially in children. [24]

Thantsha MS, Cloete TE (2006)[25] proposed that electrochemical activation (ECA) technology provides an alternative way of controlling these microorganisms and concluded that electrochemical activated solution has the potential to serve as an environmentally safe disinfectant for the control of biofilms.

Hence the present study was conducted to evaluate and compare the antibacterial efficacy of conventionally prepared electrochemically activated water and 0.2% commercially available chlorhexidine as a mouth rinse on salivary Streptococcus mutans levels in children.

Electrochemically activated solutions (ECAS) have been shown to have broad-spectrum antimicrobial activity, and have the potential to be widely adopted within the healthcare environment due to low-cost raw material requirements, ease of production, rapid disinfection time, requires little operator skill, limited toxicity, environmentally compatible and evidence of being anti-inflammatory.[5]

Electrochemical treatment in the anode and cathode chambers results in the synthesis of two types of solutions, that produced in the anode chamber is termed an anolyte; and that produced in the cathode chamber is catholyte. Anolyte solutions containing a mixture of oxidizing substances demonstrate pronounced microbiocidal effectiveness against bacteria, viruses, fungi and protozoa. Anolyte solution has been termed Super-Oxidized Water or Oxidative Potential Water. Depending on the type ECA device incorporated the FEM (Flow-through Electrolytic Module) elements the pH of anolyte varies; it may be acidic (anolyte), neutral (anolyte neutral) or alkaline (anolyte neutral cathodic).[26]

The electrochemical activated solution acts as a potent microbiocidal agent. Under clean conditions, freshly generated super-oxidized solution (ECAS) was found to be highly active against all these microorganisms giving a 99.999% or greater reduction in two minutes or less.[27,28]

The present study results showed that electrochemically activated water was highly effective in reduction of Streptococcus mutans count when used as mouth rinse. The study results were in accordance with the study done by Marais JT, Brozel VS (1999)[29]which showed that electrochemically activated water was effective in

controlling bacterial counts and total elimination of biofilm when used in dental water unit lines.

Robinson G et al (2013)[30] demonstrated that ECAS can retain basic bactericidal activity for over 12 months. The study done by Cloete TE et al (2009)[31]concluded that anolyte in electro-chemically activated water caused bacterial death by complete destruction of proteins or by causing oxidative stress which resulted in protein fragmentation. Zinkevich V et al (2000)[32] proposed that the biocidal properties of sterilox, a non-toxic liquid biocide electrochemically activated water are due to its effect upon constituents of the bacterial cell including proteins and nucleic acids.

The results of the study done by Park H et al (2004)[33] showed that the high oxidation potential of electro chemically activated solutions may inhibit microbial growth through oxidation of sulfhydryl compounds on cell surfaces and other key metabolites. The study results done by Rogers JV et al (2006)[34] demonstrated that ECA solutions have the ability to eliminate or destroy bacterial endospores.

Hence the present study suggests the use of electrochemically activated water as an environmental friendly alternative to 0.2% chlorhexidine because of its proven antibacterial efficacy.

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