Efficacy of 10% Povidone Iodine in Children Affected with Early Childhood Caries: An *In Vivo* Study

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For prevention of dental caries, S. mutans numbers must be reduced and prevented from returning to the original level. An antibacterial agent that is effective and also acceptable to young children can help to establish a favorable oral environment and halt the caries process. ***Purpose:*** *This study was conducted to evaluate the efficacy of topical antimicrobial (10% Povidone- Iodine) on S.mutans counts in children with Early Childhood Caries after full mouth rehabilitation. Also its effects on the clinical outcomes in terms of incidence of new caries and secondary caries were evaluated.*

Method: *Full mouth rehabilitation was done in 30 children (mean age 4.2 years) suffering from Severe Early Childhood Caries (SECC). Stimulated saliva samples were collected to determine the post operative baseline S.mutans counts. Thereafter the subjects were randomly divided into two groups. The experimental group received 10% Povidone Iodine at 3 months interval for a period of 12 months while the control group received placebo (deionized water) at similar intervals.*

Change in the S.mutans *count at 6 and 12 month intervals were compared to the baseline post-operative mutans score. Results revealed that application of 10% Povidone Iodine caused a significant reduction in the rise of Streptococcus mutans levels from the baseline postoperative score after 12 months of treatment thus decreasing the oral load of the organisms. Reduction in counts; in turn decreased the relapse of caries in these children.* ***Conclusion:*** *Thus oral rehabilitation coupled with regular application of 10% Povidone Iodine application can be a good alternative to control dental caries in children affected with Early Childhood Caries (ECC).*

Keywords: *Early Childhood caries, Antimicrobial, Streptococcus mutans*

J Clin Pediatr Dent 34(3): 233–238, 2010

INTRODUCTION

Early Childhood Caries (ECC) is a life style disease that begins when the child's teeth erupt in the oral cavity. Maternal factors such as high DMFS score, low education level, prolonged breast feeding and pacifier use are the risk factors for the colonization of caries related microorganisms.[1] Besides low socioeconomic status, poor oral hygiene (presence of visible dental plaque), lack of fluoride exposure and an abnormal saliva secretion rate also contribute to its etiology.

Furthermore, the importance of microbiological factors in the initiation and progression of disease has also been recognized. van Houte *et al* found that *S. mutans* formed a significantly greater proportion of the carious flora.[2] Its prevalence increases with age and the number of erupted teeth.[3] Accumulation of these organisms to pathogenic levels (>10^5 cfu/ml of saliva) is a predictor of caries.[4]

Management of ECC depends on the extent of the lesion, age of the child, behavior of the child and degree of cooperation of parents. Therapy is usually restricted to surgical removal or restoration of carious teeth coupled with recommendations regarding feeding and oral hygiene habits. The key to the problem of ECC is prevention. Besides parental counseling for infant dental care, antimicrobials have also been found to be effective.

Traditionally topical fluorides have been used as anti-plaque and anti-caries agents. They are known to inhibit the coherence of *S. mutans* at concentration as low as 0.001%.[5] However dose calculations and toxicity due to possible ingestion remains a potential problem in the children of preschool age. Chlorhexidine has also been found to reduce

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S. mutans in saliva and dental plaque although the bacterial counts eventually return to pre-treatment levels.[6] The need for frequent application of Chlorhexidine and other side effects such as unpleasant taste and staining has stimulated the search for alternatives. Although Amin *et al* could not demonstrate any significant difference in the microbial counts of children receiving placebo and 10% Povidone Iodine,[7] Zahn *et al* concluded that one time treatment with Povidone Iodine effectively reduced the microbial counts for up to 3 months.[8]

Hence this study was conducted to evaluate the efficacy of topical antimicrobial (10% Povidone- Iodine) on *S. mutans* counts in children with ECC after full mouth rehabilitation. Also its effects on the treatment outcomes in terms of incidence of new caries and secondary caries were evaluated.

MATERIALS AND METHOD

Children (mean age 4.2 years) from schools around the institution were examined by a single examiner using a plain mouth mirror, wooden tongue spatula and a gauge piece in adequate natural light. def index was recorded using WHO criteria.[9]

Out of those who suffered from ECC, subjects who satisfied the following criteria were selected for the purpose of study. Inclusion criteria: Healthy Children of age less than 5 years with only primary teeth at the 12 month follow up visit. def > age+1 (severe ECC)[10] with at least 15 teeth remaining after treatment. These children should not have consumed systemic antibiotics within 14 days of the microbial sample collection. The study was approved by the Institutional ethical committee.

Pretreatment records and saliva sample collection-

A baseline sample of stimulated whole saliva was collected between 10 am and 12 noon. Each child was asked to chew on a cotton roll for 1-2 minutes. The cotton roll was placed in the barrel of a 5 ml sterile disposable syringe and 1 ml saliva expressed by syringe plunger in a sterile test tube containing 1 ml of thioglycollate broth. The samples were then microbiologically analyzed for the presence of *S. mutans*.

A thorough clinical examination was carried out to record the def.

Treatment procedures

Thorough dental prophylaxis and oral rehabilitation was done depending on the treatment needs of the patient under local anesthesia. Mainly composite, amalgam, stainless steel and Strip crowns were used. No fluoride releasing material was used in this study.

Immediate Post treatment records and saliva collection-

Immediately after the treatment was considered complete (same appointment), saliva samples were again collected using the above mentioned technique and analyzed for *S. mutans* counts.

Subjects were requested to pick any coin from a bowl that contained equal number of (15 each) blue and red coins. All those who had chosen red coin were allocated to experimental group while the blue coin pickers were controls. The subjects were unaware of their allocation to the respective groups.

Experimental group- Subjects in this group received four applications of topical antimicrobial (Povidone-Iodine 10%) at 3 month intervals after complete oral prophylaxis and rehabilitation.

Control group- Subjects in this group received four applications of placebo (Deionized water) at three month interval after complete oral prophylaxis and rehabilitation.

The teeth were dried and isolated with cotton rolls. A solution of 10% Povidone Iodine (containing 1% active iodine) was applied for 4-5 minutes using a small sterile saturated cotton ball held in locking cotton pliers. A total of 0.20 ml of iodine solution was used for each treatment- a dose that contains 2 mg of iodine, which is nontoxic when applied. The treated individuals received a total of four iodine applications at interval of 3 months during the study period.

Deionized water was applied to the dentition of the subjects in the control group by a similar technique and at similar intervals.

At each follow up examination, about 1 ml of saliva sample was collected for every child and subjected to microbiological analysis for *S. mutans* colony counting.

The saliva samples were dispersed for 10 s by sonification and plated in duplicate by means of an automatic diluting and plating device on TYCSB agar selective for *S.mutans*. The plates were incubated in Anerogas Pack (Hi Media Laboratories Ltd, Mumbai, India) containing jars at 37^0 C for 48 hours. Only those colonies which revealed *S.mutans* characteristics[11] were counted using a colony counter by a laboratory investigator who had no knowledge about the clinical status of the subjects. Mannitol and sorbitol fermentation tests were used for biochemical confirmation.

Treatment evaluation and follow up after 6 and 12 months

Relapse was defined as the presence of 1 or more new smooth surface caries lesion(s) affecting any primary tooth or teeth.[12] A carious lesion that develops at the interface of the restoration and the cavosurface of enamel is called recurrent caries. Secondary caries was adjudged to be presented only if there was a frank carious cavity (defect>0.5mm) next to margin of the restoration.[13]

RESULTS

All children returned to their recall appointments.

Table 1 reveals the number of *S.mutans* per ml of saliva in children of control and the experimental group.

All the data were transformed to $\log_{10}$ to stabilize the

Table 1. *Streptococcus mutans* counts/ ml of saliva of children of control and experimental group

	Control group					Experimental group			
No.	Pre operative counts	Post operative counts	Counts after 6 months	Counts after 12 months	No.	Pre operative counts	Post operative counts	Counts after 6 months	Counts after 12 months
1	500000	25000	250000	500000	1	250000	25000	125000	250000
2	50000000	50000	50000	75000	2	50000000	150000	125000	500000
3	50000000	10000	50000000	50000000	3	50000000	0	25000	25000
4	50000	25000	250000	50000000	4	75000	25000	12500	25000
5	50000000	125000	50000000	75000	5	50000	25000	250000	25000
6	50000000	125000	500000	50000000	6	500000	0	10000	250000
7	50000000	1000	250000	50000000	7	500000	50000	250000	50000000
8	50000000	75000	250000	50000000	8	50000000	250000	350000	500000
9	750000	100000	12500	50000	9	50000000	50000	25000	500000
10	50000000	120000	350000	50000000	10	50000000	25000	250000	500000
11	500000	5000	50000	75000	11	50000000	250000	500000	750000
12	50000000	100000	250000	750000	12	250000	2500	12500	100000
13	50000000	25000	125000	500000	13	50000000	50000	250000	500000
14	50000000	250000	500000	50000000	14	50000000	25000	12500	250000
15	50000000	25000	250000	50000000	15	10000	5000	1500	5000

variation within and between subsets of data. Pre and post treatment *S.mutans* counts obtained with and without the use of antimicrobial were compared using non parametric Wilcoxon sign rank test.

Results revealed that a statistically highly significant fall in the bacterial counts took place in all the subjects following oral rehabilitation (p=0.001). (Graph 1)

Differences in log $_{10}$ counts of *S. mutans* after treatment (post operative score), 6 months and 12 months were compared for experimental and control group using t test.

At 6 month follow up visit there was a mean rise of 68.02 in *S. mutans* counts from the post operative score in the control group compared to 0.84 in the children receiving topical antimicrobial. However the difference in the rise of bacterial counts of two groups was not statistically significant (p=0.1485). (Table 2; graph 1)

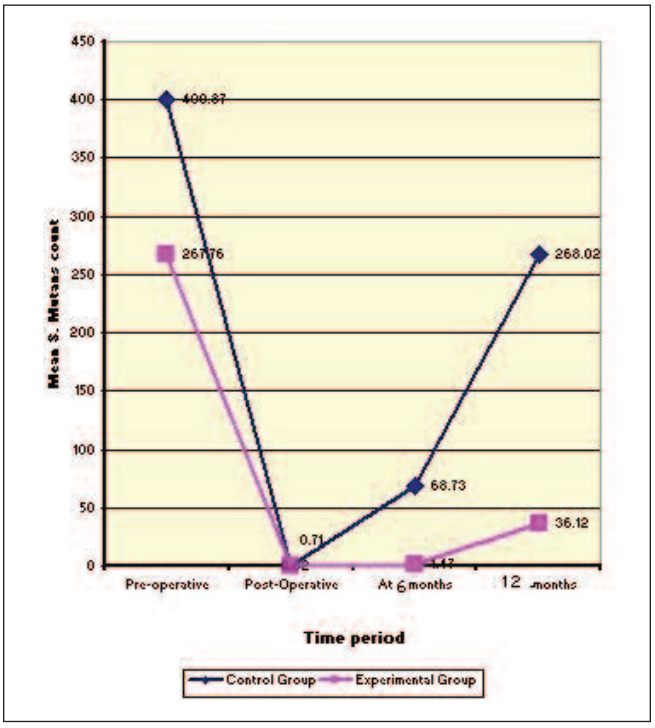
At 12 month follow up visit there was a mean rise of 267.31 in *S.mutans* counts from the post operative score in the control group compared to 35.5 in the children receiving topical antimicrobial. The difference in the rise of bacterial counts of two groups was statistically significant (p=0.0041) (Table 2; graph 1) pointing out to the role of Povidone Iodine in suppressing the re-growth of these bacteria.

Clinically 2 children of the control group revealed new carious lesions on the smooth surfaces. Statistically the difference was not statistically significant in the children of the control and the experimental group. (Table 3; graph 2)

No secondary carious lesion was found in any subject.

Table 2. Comparison of the change in Streptococcus mutans counts after full mouth rehabilitation in the saliva of children receiving topical antimicrobial and placebo in a 12 month perspective. (To determine the effect of antimicrobial application)

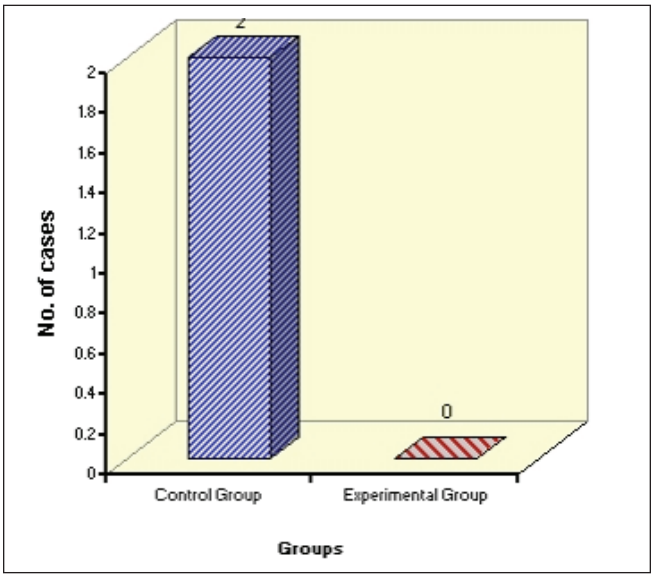
	Control group		Experimental group			
Follow up time	Mean change in count	Standard deviation	Mean change in count	Standard deviation	t value	p value
6 months	68.02	175.11	0.84	1.07	1.49	0.1485
12 months	267.31	256.62	35.5	128.38	3.13	0.0041



Graph 1.

Table 3. Comparison of relapse of caries over 12 months in control group and experimental group.

Follow up time	Number of cases that presented with relapse in Control group	Number of cases that presented with relapse in Experimental group	Chi square value	p value
6 months	0	0	2.678	0.10174
12 months	2	0		



Graph 2.

DISCUSSION

Povidone iodine (PVP-I) or betadine is a potent microbicidal agent. The 10% PVP-I solution contains 90% water, 8.5% polyvinyl pyrrolidone (PVP) and only 1% available iodine. PVP, the hydrophilic polymer that acts as a carrier, does not have any intrinsic antibacterial activity but reduces irritability, and decreases the staining caused by pure iodine. Because of its affinity to the cell membrane it delivers free iodine (I_2) directly to the bacterial cell surface. Iodine targets are located in the bacterial cytoplasm and cytoplasmic membrane, and its cidal action takes place in a matter of seconds.[7]

Literature review reveals a very few studies that present the evidence for the effectiveness of Povidone Iodine in prevention of dental caries in 'high risk' children.[7,8,14] Although well designed, none of the studies identified and controlled for factors such as socioeconomic status of the participants, dietary habits, fluoridation status of the residential areas and schools of the participants, dental visits, or use of topical or systemic antimicrobials. Though difficult to balance in a randomized controlled clinical trial, these confounders might have a significant influence on the clinical outcomes of the study. So as to minimize the loss to follow up and compensate for at least few confounding factors; 30 children from a near by school who suffered from SECC were treated and followed up. This ensured that the children resided in the same geographic area, were exposed to more or less same fluoride levels and belonged to similar socioeconomic strata. Besides, the dietary pattern, during the school hours could also be more or less equated.

The bacterial species found to be over abundant in caries active group are *Actinomyces, Streptococcus mutans* and *Lactobacillus species*.[15] In this study, the value of experimental agents was measured in terms of their effect *S. mutans*. Since *S. mutans* primarily colonizes the teeth and is present in much lower proportions on oral mucosal surfaces, it is reasonable to assume that most of the *S. mutans* cells present in the saliva are derived from bacterial plaque present on the teeth. Thus for the purpose of this study stimulated saliva samples were collected which can be considered as an 'average' for the individual oral load of *Mutans Streptococci*. Caufield *et al* [16] found a significant correlation between the levels of *S. mutans* in saliva and proportions of this organism in plaque.

Studies reveal that *MS* and *lactobacilli* counts performed on saliva samples collected before breakfast and brushing were substantially higher than those collected at other times during the day.[17] Thus to over come these variations all the saliva samples were collected before the children could have their mid day snack. This also ensured a gap of 1 or 2 hours after breakfast.

Immediate post operative microbiological testing of the saliva sample revealed a highly significant reduction in the counts of *S. mutans* in both control and the experimental group(Table 1,2; graph 1). The finding that microbial levels dropped significantly immediately after extensive operative and restorative treatment was attributed to the decreased number of retention sites due to restorations and extractions.

It is noteworthy that even after thorough oral prophylaxis and restorative treatment the bacterial counts did not fall to absolute zero immediately after treatment. This is in accordance with previous study of Twetmen *et al* [18] who emphasized that approximately 50 % of the children with a few extracted teeth and mainly restorative care still exhibited high counts of *Mutans Streptococci* after 1 and 6 months of treatment. Gregory *et al* [19] found no significant differences in pre to post restoration numbers of total oral *Streptococci*. The return of *S. mutans* at a particular site to its original level can be explained on the basis of outgrowth of a few remaining viable cells at that site or by reinfection *via* the saliva.[20]

Also in this study the observation might be attributable to the fact that treatment for all these children was carried out over multiple visits using behavior modification techniques rather than a single visit under general anesthesia as compared to other studies. Retention sites such as occlusal fissures, enamel cracks, or micro spaces between the dental restorations and the cavity walls of meanwhile untreated teeth might have caused regrowth of *Streptococcus mutans* during the interval for next appointment. Thus by the time all the teeth were restored; some regrowth of *S. mutans* might have taken place.

Bowden *et al* suggested that the stage of development of

oral biofilms could also influence the release of *S. mutans* and *S. sanguis* into the planktonic phase (saliva) and that early developing biofilms liberated more cells owing to the less retention of these organisms in the biofilm during the growth phase.[21] This might be another cause of recovery of *S. mutans* from the saliva after restorative treatment.

According to the Ecological plaque hypothesis, along with the alteration of the oral environment, bacterial species must either be eliminated from the oral cavity or when reduced must be prevented from returning to its pathological level to prevent dental caries. An antibacterial agent that is effective and also acceptable to young children can help to establish a favorable oral environment and stop the disease process.

Chlorhexidine and Sodium fluoride have been used as traditional anti bacterial agents. However the uptake and release of Chlorhexidine is highly pH dependent (uptake is reduced at low pH) and is also inhibited by calcium ions. Frequent application and taste also make its use objectionable to many children. Fluorides on the other hand exhibit the problem of ingestion and toxicity. Thus 10% Povidone-Iodine containing 1 % active iodine was used.

A total of 0.20 ml of iodine solution was used for each treatment- a dose that contains 2 mg of iodine, which is non-toxic when applied. The solution was applied for 4-5 minutes. This is in accordance with the study of Tanzer *et al* [22] who found that Povidone-iodine was bactericidal at a concentration of 1% titratable iodine for *S.mutans* and *A.viscosus*, but was bacteriostatic for *S.sanguis* and *A.naeslundii*. The minimum time required for killing *S.mutans* and *S.sanguis* was 5 minutes while *A viscosus* and *A naeslundii* required a minimum of 30 minute application.

None of the studies conducted before actually determine the role of antimicrobial in delaying the regrowth /re establishment of the bacteria in children. The immediate post operative counts reflect the minimum load of microorganisms in the oral cavity. To evaluate the effect of antimicrobial, *S. mutans* counts after 6 and 12 months of treatment were compared to the immediate post operative counts. This comparison reflected the effectiveness of antimicrobial in actually delaying the re-establishment of the offending organism in the oral cavity.

Results indicate that there was no significant difference in the change of *S. mutans* counts after 6 months compared to the immediate post operative score in both the control and experimental groups. However there is a statistically highly significant rise in the *Streptococcus mutans* counts after 12 months in the control group compared to the experimental group; thus pointing out to the long term role of 10% Povidone- Iodine in preventing bacterial re-establishment.

Chase *et al* [23] concluded that although dental surgery led to a significant reduction in salivary *Mutans Streptococci* reservoirs, the relapse rate remained to be high and rapid for children treated for ECC.

Although not statistically significant, in this study 2 subjects in the control group revealed new decalcified carious lesions on the smooth surfaces. (Table 3, Graph *2)*. This in accordance with the previous study of Amin *et al* [7] which at 6 months interval revealed that 3 children in the control group and 1 in the experimental group demonstrated newly decalcified surfaces.

Caufield *et al* [24] found that I_2-KI solution was more effective in reducing the mean buccal caries score while Sodium fluoride was found to be more effective for sulcal sites. The inability of iodine to reduce fissure caries possibly reflected of its inability of iodine to be retained in the sulcus, its lack of penetration to the depth of microbial mass, and tendency of sulcus to be quickly recolonized following disinfection. Accordingly it may be presumed that selective action of Povidone –Iodine on smooth surfaces makes it a beneficial antimicrobial agent to be used in children suffering from ECC where smooth surfaces are the most susceptible sites.

To conclude, in developing countries that have limited means, more cost effective measures undertaken to eradicate disease are imperative. Its ease of availability and cost effectiveness coupled with benefits of rapid antimicrobial activity and decreased adverse effects (except for few reports of hypersensitivity) indeed make Povidone- Iodine a chemotherapeutic agent of choice in home regimes.

Certain uncontrolled external factors such as oral hygiene behavior of children and dietary habits might have affected the results. To overcome these confounders the study design can be modified to a crossover design where by the children after oral rehabilitation could be followed up for a period of time and then the same children could receive topical antimicrobial thereafter. Furthermore the wash out effect of Povidone-Iodine after its withdrawal can be studied. Costs and benefits of treatment with Povidone-Iodine compared to other traditional antimicrobial agents also warrant further research.

Further studies can be done on a larger population for a longer follow up period with minimum subject drop outs to assess the duration, frequency and different concentrations of Povidone-Iodine; to identify the best possible concentration that can significantly improve the clinical outcomes in children affected with ECC.

CONCLUSIONS

Application of 10% Povidone Iodine leads to a significant reduction in rise of *Streptococcus mutans* counts from the baseline postoperative score in experimental group compared to the control group after 12 months of treatment. Thus oral rehabilitation coupled with regular application of 10% Povidone Iodine application can be a good alternative to control dental caries in children affected with ECC.

ACKNOWLEDGEMENTS

Our sincere thanks to Department of Microbiology, Christian Medical College, Ludhiana, Punjab, India for their unflinching support during the course of the study. We also acknowledge the efforts of our statistician Mr. Gurinderjit Singh (Guru Nanak Dev University, Amritsar, India) for the data analysis.

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