

Comparative Evaluation of Antimicrobial Efficacy of Silver Diamine Fluoride and Silver Diamine Fluoride with Potassium Iodide on *Candida albicans* and Lactobacilli Count and Effect on Salivary pH in Children- An In-Vivo Study

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DOI: 10.47750/pnr.2022.13.S07.113

Abstract

Background : Dental caries is described as the localized destruction of susceptible dental hard tissues caused by acidic by-products of microbial fermentation of dietary carbohydrates. **Aim :-** The aim of this study is to evaluate and compare the efficacy of Silver Diamine Fluoride and the combination of Silver Diamine Fluoride with Potassium Iodide on the *Candida albicans* and Lactobacillus count and pH of saliva in children with carious deciduous molars. **Method:** The study involved 60 children from the age group of 3 to 9 years were randomly divided into 2 groups, Group I- Silver Diamine Fluoride and Group II- Silver Diamine Fluoride and Potassium Iodide. Each group was further divided into subgroups of *Candida albicans* and Lactobacilli Group. The Saliva samples were collected from each candidate before and after the application of the material to check the microbial count as well as the pH of the saliva as a baseline value. The post application saliva collection as well as pH check was done at the interval of Immediate after the application, 1 week, 1month and 3 months. The results obtained were analysed using ANOVA, and student t test. **Result:** The pH of the saliva was increased after the application of both the materials and was effective till 3 months. Both the materials were effective in decreasing the microbial count. **Conclusion:** The result of the study confirms that both Silver Diamine Fluoride and Silver Diamine Fluoride and Potassium iodide have antimicrobial effect on both *Candida albicans* and lactobacilli species.

INTRODUCTION

Dental caries is initially reversible and can be stopped at any point, even if some dentine or enamel has been damaged (cavitation), as long as enough biofilm is eliminated According to Becker et al, in 2002 some species, such as Streptococcus species, Veillonella species, Actinomyces species, Bifidobacterium species, and Lactobacillus fermentum, have been linked to caries⁽²⁾. Children with early childhood caries had more candida spp. in their saliva, dental plaque, and infected dentine than children without caries.⁽³⁾ *Candida albicans* are associated with early childhood caries⁽⁴⁾ The most common species of lactobacilli which are associated with dental caries are *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus rhamnosus*, *Lactobacillus salivarius* etc⁽⁵⁾. Traditional restorative or surgical approaches to caries, are being challenged by more biological, less invasive approaches to arrest carious lesions (Ricketts et al., 2013; Schwendicke et al., 2013).⁽⁶⁾ A new product like Silver Diamine Fluoride is being used as a cariostatic agent against bacteria like Streptococci, Actinomyces, and Lactobacilli⁽⁷⁾. Due to the interaction of free silver ions with organic material, topical administration of SDF

stained organic material found in pellicle and active caries. Knight et al. in 2005 proposed a new technique to solve this problem by administering a saturated potassium iodide (KI) solution immediately after SDF administration⁽⁸⁾. This paper aims to evaluate and compare the efficacy of Silver Diamine Fluoride and the combination of Silver Diamine Fluoride with Potassium Iodide on the *Candida albicans* and Lactobacillus count and pH of saliva in children with carious deciduous molars

METHODS

A total of 87 Patients in the age group of 3 yrs to 9 yrs were screened. A written consent form from the respective parents of the children were taken.. Using Microsoft Excel software, the patients were assigned codes and the participants were randomly split into 2 groups, Group I- Silver Diamine Fluoride and Group II- Silver Diamine Fluoride with Potassium Iodide. Each group was further divided into subgroups of *Candida albicans* and Lactobacilli Group. Healthy patient age ranging from 3-9 years with multiple carious deciduous teeth were selected. 2ml of unstimulated saliva was collected by drooling method and the samples were coded. During subsequent saliva sample collection, the same code number was used for the same patient. The pH strips (Indikrom pH strips) were placed in the obtained unstimulated saliva sample for 10 seconds to measure the salivary pH. In group I, 38% SDF was applied directly to the tooth carious lesion and was kept on for about 4 minutes. Cotton roll was used to remove excess SDF. In Group II, After the application of SDF, the freshly prepared 20% Potassium iodide solution was applied on the carious part using a new sterile micro brush. After around 4-minute cotton roll was used to remove the excess. Saliva sample was again collected. The sample collected were sent to the microbiological laboratory for *Candida albicans* and Lactobacilli counts. To preserve the viability of the test organism, the samples were kept at a temperature of -20°C. The saliva samples were inoculated on Hi chrome differential agar plate to cultivate for *Candida albicans*. For 72 hours, the plates were incubated at 37°C. To cultivate Lactobacilli, the salivary samples were inoculated on Rogosa Agar plates and incubated in a 5% CO₂ incubator at 37°C for 48 hours. Microbial count were estimated as colony forming units (CFU/ ml) in saliva. Similarly, saliva samples from 24 hours, one week, one month, and three months were collected and the results were obtained.

STATISTICAL ANALYSIS: Data obtained were analysed using Student t test and ANNOVA test.

RESULT: When the mean *Candida albicans* count was compared among the groups, it was found that there was no statistically significant difference between the two groups at different time intervals ($p > 0.05$). (Table 1) The mean salivary Ph had no statistically significant difference between the two groups at different time intervals ($p > 0.05$). In both the groups, the pH remained neutral after applying SDF or SDF with KI.(Table 2) The mean lactobacillus count also showed no statistically significant difference between the two groups at different time intervals ($p > 0.05$). (Tble 3) Similarly the the mean salivary pH (lactobacilli) also showed no statistically significant difference between the two groups at different time intervals ($p > 0.05$). (Table 4)

Table 1: Comparison of mean *Candida albicans* count among the two groups at different time intervals

Time	SDF		SDF with KI		p-value
	Mean	S.D.	Mean	S.D.	
Baseline	39.87	38.27	38.60	27.99	0.918
Immediately	1.73	2.66	2.07	2.63	0.733
1 week	4.73	5.27	4.67	3.69	0.968
1 month	8.73	7.69	9.27	5.58	0.830
3 months	16.73	13.32	17.13	10.99	0.929

Table 2: Comparison of mean salivary pH among the two groups at different time intervals

Time	SDF		SDF with KI		p-value
	Mean	S.D.	Mean	S.D.	
Baseline	6.80	0.414	6.67	0.488	0.426
Immediately	7.00	0.0	7.00	0.0	-
1 week	7.00	0.0	7.00	0.0	-
1 month	7.00	0.0	7.00	0.0	-
3 months	7.00	0.0	7.00	0.0	-

Student t test applied, p-value non-significant

Table 3: Comparison of mean lactobacillus count among the two groups at different time intervals

Time	SDF and KI		SDF		p-value
	Mean	S.D.	Mean	S.D.	
Baseline	120.87	83.39	97.93	92.18	0.481
Immediately	2.13	3.11	1.80	2.73	0.758
1 week	6.27	6.45	4.87	6.17	0.549
1 month	11.67	9.36	9.40	8.71	0.498
3 months	22.47	18.26	18.00	15.95	0.481

Student t test applied, p-value non-significant

Table 4: Comparison of mean salivary pH (lactobacilli) among the two groups at different time intervals

Time	SDF		SDF with KI		p-value
	Mean	S.D.	Mean	S.D.	
Baseline	6.47	0.516	6.47	0.516	1.00
Immediately	7.00	0.0	7.00	0.0	1.00
1 week	7.00	0.0	7.00	0.0	1.00
1 month	7.00	0.0	7.00	0.0	1.00
3 months	7.00	0.0	7.00	0.0	1.00

Student t test applied, p-value non-significant

DISCUSSION

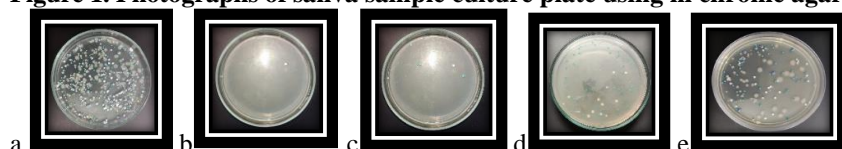
Dental caries is caused by the demineralization of the hydroxyapatite crystal of the teeth by the acid by-products produced by the bacteria in the dental biofilm. According to Human Oral Microbiome database, oral cavity comprises of about 600 species of microbes. **Kleinberg et al. in 2002** suggested that the main culprit of the caries are the acid producing bacteria. ⁽¹²⁾ Many studies have shown that Streptococcus are the main causative bacteria for caries followed by lactobacilli species and Actinomyces species. ^{(13) (14) (15)} In 2008, **Marsh and Nyvad et al.** reported that the acid produced from the fermentation of the carbohydrate by the oral microbes further enhance the demineralization of the teeth. As the oral pH decrease the harmony between the host and the bacteria is disrupted and gets changed into a parasitic relationship (**Stanier et al., 1970**). During the early colonization stage, Mutan Streptococci species were more predominant which indicate the initialization of the carious process whereas on the advancing front many bacterial species like Lactobacillus, Actinomyces, non-mutans streptococci, Bifidobacterium and Propionibacterium help in progression of the carious lesion (**Aas et al., 2005, 2008**). ⁽¹²⁾ Among the Lactobacilli species, Lactobacillus fermentum were found in higher number in the children affected with early childhood caries or with carious lesion (**Svec et al., 2009; Piwat et al., 2010; Tanner et al., 2011; Teanpaisan et al., 2012; Caufield et al., 2015**). The potent cariogenic nature of Lactobacilli species can be attributed to its acidogenic properties as well as the ability of strong binding with Type I collagen as dentine contain higher amount of type I collagen (**Aleljung et al., 1991; McGrady et al., 1995**). ⁽¹⁶⁾ In 2000, **Williams DW et al.**, identified about 20 species of Candida that are found in human. ⁽¹⁷⁾ Among all the species of the Candida, Candida albican is the most commonly found species and can be mostly obtained from different areas in the oral cavity. It was reported that Candida had the ability to cause dental caries due to their higher potential of causing demineralization of hydroxyapatite crystal and also increase the colonization of the caries causing streptococcus mutan species (**Marchant S et al. 2001; Shen S Samaranayake LP et al. 2002; Barbieri DSE et al. 2007**). ⁽¹⁸⁾ The buffering and clearance capacity of saliva depends on the flow rate of the saliva (**Birkhed and Heintze, 1989; Miura et al., 1991**). ⁽¹⁹⁾ In stimulated saliva the proportion of Bacilli varied from 15 to 40%, whereas in the unstimulated saliva they exceeded 50% (**Gomar-Vercher S et al. 2018**). ⁽²⁰⁾ According to the study conducted by **Rozkiewicz D et al (2006)** ⁽²¹⁾ the prevalence of oral candidal carriage is strongly linked with the incidence of dental caries in children of 4 to 7 yrs old. So, 4 to 9 yrs. groups were selected for the study. The unstimulated saliva was frequently selected over the stimulated saliva as stimulated saliva contained a diluted quantity of biomarkers, which may be difficult to detect (**Daniel Malamud D et al in (1992)** ⁽²²⁾ **Beighton et al. in 1995** ⁽²³⁾, had proven the use of Hi CHROME agar plates being a deferential media would significantly enhance the study of the oral yeast flora. 38% Silver diamine Fluoride was utilised in this study in accordance to **R.yee C. Holmgren et al. (2009)** ⁽²⁴⁾ 38% Silver diamine Fluoride had the highest antimicrobial efficacy when compared with different concentration. In the present study along with Candida albican, the efficacy of Silver diamine Fluoride and Silver diamine fluoride on lactobacilli species were also considered. Lactobacilli species were selected as they are the causative organism of dental caries in accordance with the study of **Ademe D et al in 2020**. ⁽²⁵⁾ To calculate the colony forming unit for Lactobacilli species, a selective media- Rogosa SL was employed in accordance with the study conducted by **Rogosa et al in 1951** ⁽²⁶⁾ In this study, the mean age of the candida group patients was 6.27 ± 1.59 years and the lactobacilli group was 6.00 ± 1.20 years with higher prevalence in female with 63.3% among candida group and 56.7% among the lactobacilli group. This result was in accordance with **Raja M et al in 2007** ⁽²⁷⁾ where they had

found that there was higher prevalence of candida in female than male children. In this study it was found that the mean *Candida albican* count was drastically reduced to 1.73 ± 2.66 immediately after applying SDF therapy. The count gradually increased from baseline to 1 month and then 3 months reaching at 16.73 ± 13.32 . This result was in accordance with the study conducted by **Fakhruddin et al in 2020**,⁽²⁸⁾ The profound reduction in the *Candida albican* count after the application of Silver Diamine Fluoride may be attributed to the fact that SDF had broad spectrum antimicrobial activity against the fungi and bacteria.⁽²⁴⁾ The capacity of the Silver Diamine Fluoride to inhibit the formation and adhesion of plaque bacteria may also be another contributing factors for its antimicrobial effect.⁽²⁹⁾ To control the black staining effect of Silver diamine fluoride, 20% Potassium iodide was used in accordance with study by **Nguyen et al in 2017**⁽¹¹⁾ In our study there was 80% reduction in *Candida albicans* count when Silver Diamine Fluoride with Potassium Iodide were used whereas 60% reduction of *Candida albicans* count were noted in Silver Diamine group. This studies goes in accordance with **Moalic et al., 2001**⁽³⁰⁾ and **Rozkiewicz et al., 2006**⁽²¹⁾ After the application of Silver Diamine Fluoride with Potassium iodide, the count of the Lactobacilli species was 120.87 ± 83.39 at baseline which drastically reduced to 2.13 ± 3.11 immediately after applying SDF and KI. The count gradually increased from baseline to 1 month and then 3 months reaching at 22.47 ± 18.26 . The mean lactobacillus count among the two groups at different time intervals had no statistically significant difference. Since no other studies were found which compare the efficacy of Silver Diamine Fluoride and Silver Diamine Fluoride with Potassium Iodide on lactobacilli, further studies would be necessary to determine the potential of these anticariogenic materials. In this study it was shown that percentage of lactobacilli cases among the two groups were found to be more in SDF and KI group at different time intervals when compared with SDF group. This result was in accordance with **Robert A et al n 2020**⁽³¹⁾ where they found that when compared to SDF alone, SDF+KI was found to be the most efficient at inhibiting *S mutans* migration through dentine. Since no studies were found that corelate the antimicrobial efficacy of SDF and KI on Lactobacilli. Further studies are required to prove its efficacy against these microbes.

CONCLUSION

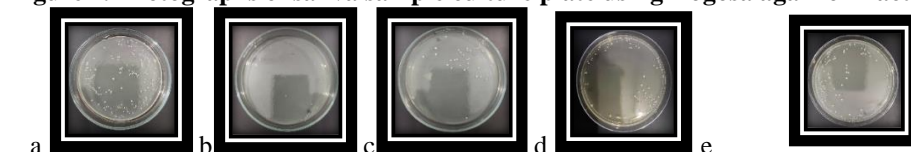
In conclusion it was found that Silver Diamine Fluoride are effective against *Candida albican* and Lactobacilli species and also effective in increasing the salivary pH. It was also found that when Potassium iodide is applied after Silver Diamine Fluoride application, the staining/ darkening effect of the agent is drastically reduced. Potassium iodide does not hamper the antimicrobial and antifungal activity of the Silver Diamine Fluoride. Silver Diamine Fluoride and Potassium Iodide also increases the salivary pH after the application. Thus, Silver Diamine Fluoride and the combination of Silver Diamine Fluoride with Potassium Iodide are equally effective in decreasing the count of *Candida albicans* and Lactobacilli species and also equally effective in increasing the salivary pH in children with carious deciduous molars.

Figure 1. Photographs of saliva sample culture plate using hi chrome agar for *Candida albican*



a. Baseline salivary sample, b. Immediate post op saliva sample, c. 1 week post op saliva sample
d. 1 month post op saliva sample, e. 3 months post op saliva sample

Figure 4. Photographs of saliva sample culture plate using Rogosa agar for Lactobacilli species



a. Baseline salivary sample, b. Immediate post op saliva sample, c. 1 week post op saliva sample
d. 1 month post op saliva sample, e. 3 months post op saliva sample

BIBLOGRAPHY

- Selwitz RH, Ismail AI, Pitts NB. Dental caries. The Lancet. 2007;369(9555):51-9.
- Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, et al. Bacteria of Dental Caries in Primary and Permanent Teeth in Children and Young Adults. 2008;46(4):1407-17.
- Klinke T, Kneist S, de Soet JJ, Kuhlisch E, Mauersberger S, Förster A, et al. Acid production by oral strains of *Candida albicans* and lactobacilli. 2009;43(2):83-91.
- Beena M, Peedikayil FC, GufranAfmed M, Chandru T, Soni K, Dhanesh NJJoISoP, et al. Comparison of *Candida* species isolated from children with and without early childhood caries: A descriptive cross-sectional study. 2017;35(4):296.

5. Caufield P, Schön C, Saraithong P, Li Y, Argimón SJ*Jodr*. Oral lactobacilli and dental caries: a model for niche adaptation in humans. 2015;94(9_suppl):110S-8S.
6. Zhao IS, Mei ML, Burrow MF, Lo EC, Chu CH. Effect of Silver Diamine Fluoride and Potassium Iodide Treatment on Secondary Caries Prevention and Tooth Discolouration in Cervical Glass Ionomer Cement Restoration. *International journal of molecular sciences*. 2017;18(2).
7. Hamama H, Yiu C, Burrow MJ*Adj*. Effect of silver diamine fluoride and potassium iodide on residual bacteria in dentinal tubules. 2015;60(1):80-7.
8. Galui S, Pal S, Pabale SL, Saha S, Sarkar SJJ*JoPR*. Stretching new boundaries of caries prevention with silver diamine fluoride: A review of literature. 2018;3(1):1.
9. Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. *Journal of dental research*. 2011;90(3):294-303.
10. Kianoush N, Adler CJ, Nguyen K-AT, Browne GV, Simonian M, Hunter N. Bacterial profile of dentine caries and the impact of pH on bacterial population diversity. *PLoS One*. 2014;9(3):e92940-e.
11. Loesche WJ, Grenier E. Detection of *Streptococcus mutans* in plaque samples by the direct fluorescent antibody test. *Journal of dental research*. 1976;55:A87-93.
12. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. *Microbiological reviews*. 1986;50(4):353-80
13. Lapidrattanakul J, Nomura R, Okawa R, Morimoto S, Tantivitayakul P, Maudcheingka T, et al. Oral Lactobacilli Related to Caries Status of Children with Primary Dentition. *Caries Res*. 2020;54(2):194-204.
14. Williams DW, Lewis MA. Isolation and identification of *Candida* from the oral cavity. *Oral Dis*. 2000;6(1):3-11.
15. Al-hebshi N, Abdulhaq A, Quadri M, Tobaigy F. Salivary carriage of *Candida* species in relation to dental caries in a population of Saudi Arabian primary school children. *The Saudi Journal for Dental Research*. 2014;6.
16. Lenander-Lumikari M, Loimaranta V. Saliva and dental caries. *Advances in dental research*. 2000;14:40-7
17. Gomar-Vercher S, Simón-Soro A, Montiel-Company JM, Almerich-Silla JM, Mira A. Stimulated and unstimulated saliva samples have significantly different bacterial profiles. *PLoS One*. 2018;13(6):e0198021.
18. Qi Q, Hu T, Zhou XJJ*oop, medicine*. Frequency, species and molecular characterization of oral *Candida* in hosts of different age in China. 2005;34(6):352-6.
19. Malamud D. Saliva as a Diagnostic Fluid. *BMJ (Clinical research ed)*. 1992;305:207-8.
20. Beighton D, Ludford R, Clark DT, Brailsford SR, Pankhurst CL, Tinsley GF, et al. Use of CHROMagar *Candida* medium for isolation of yeasts from dental samples. *Journal of clinical microbiology*. 1995;33(11):3025-7.
21. Barbieri DdSAV, Vicente VA, Fraiz FC, Lavoranti OJ, Svidzinski TIE, Pinheiro RLJB*JoM*. Analysis of the in vitro adherence of *Streptococcus mutans* and *Candida albicans*. 2007;38:624-31.
22. Ademe D, Admassu D, Balakrishnan S. Analysis of salivary level *Lactobacillus* spp. and associated factors as determinants of dental caries amongst primary school children in Harar town, eastern Ethiopia. *BMC Pediatrics*. 2020;20(1):18
23. Rogosa M, Mitchell JA, Wiseman RFJJ*odr*. A selective medium for the isolation and enumeration of oral lactobacilli. 1951;30(5):682-9
24. Raja M, Hannan A, Ali KJ*Cr*. Association of oral candidal carriage with dental caries in children. 2010;44(3):272-6.
25. Fakhruddin KS, Egusa H, Ngo HC, Panduwawala C, Pese S, Venkatachalam T, et al. Silver diamine fluoride (SDF) used in childhood caries management has potent antifungal activity against oral *Candida* species. *BMC microbiology*. 2020;20(1):95.
26. Alshahni RZ, Alshahni MM, Hiraishi N, Makimura K, Tagami J. Effect of Silver Diamine Fluoride on Reducing *Candida albicans* Adhesion on Dentine. *Mycopathologia*. 2020;185(4):691-8
27. Nguyen V, Neill C, Felsenfeld J, Primus CJH. Potassium iodide. The solution to silver diamine fluoride discoloration? 2017;5(1):555655.
28. Roberts A, Bradley J, Merkley S, Pachal T, Gopal J, Sharma DJ*Adj*. Does potassium iodide application following silver diamine fluoride reduce staining of tooth? A systematic review. 2020;65(2):109-17