

# Effect of Probiotic Containing Ice-cream on Salivary Mutans Streptococci (SMS) Levels in Children of 6-12 Years of Age: A Randomized Controlled Double Blind Study with Six-months Follow Up

DEVASYA ASHWIN<sup>1</sup>, VIJAYAPRASAD KE<sup>2</sup>, MAHANTHESH TARANATH<sup>3</sup>,  
NAVEEN KUMAR RAMAGONI<sup>4</sup>, ASHA NARA<sup>5</sup>, MYTHRI SARPANGALA<sup>6</sup>

## ABSTRACT

**Introduction:** To evaluate the caries risk based on the salivary levels of *Streptococcus mutans* in children of 6-12 years of age group before and after consuming probiotic ice-cream containing *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* La-5.

**Materials and Methods:** A double blind, placebo controlled trial was carried out in 60 children aged between 6 to 12 years with zero decayed, missing, and filled teeth (DMFT). They were randomly divided into two equal groups. Saliva sample were collected before the consumption of ice-cream and *Streptococcus mutans* count was calculated and recorded as baseline data. For the next seven days both the groups were given ice creams marked as A and B. Saliva samples were collected after ice-cream consumption at the end of study period and also after a washout period of 30 days and again after six months. Samples were inoculated and colonies were counted.

**Results:** On statistical evaluation by students paired t-test, probiotic ice-cream brought significant reduction in the *Streptococcus mutans* count after seven days of ice-cream ingestion ( $p < 0.001$ ) and also after 30 d of washout period ( $p < 0.001$ ). There was no significant reduction ( $p = 0.076$ ) by normal ice-cream consumption. After six months of the study period in both the groups the salivary levels of *Streptococcus mutans* was similar to the baseline.

**Conclusion:** Probiotic ice-cream containing *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* La-5 can cause reduction in caries causative organism. The dosage of the probiotic organisms for the long term or synergetic effect on the oral health are still needed to be explored.

**Keywords:** Mitis salivarius agar, Ice-cream, Probiotics, Saliva, *Streptococcus mutans*

## INTRODUCTION

A well known fact is that dental caries is the main oral health problem in industrialized countries, and it affects 60-90% of children and adults [1,2]. Over the years, studies confirmed that *Streptococcus mutans* (MS) is one of the most common causes for dental caries in humans [3]. This bacteria can rapidly metabolize dietary sugars to acid, creating locally a low pH. Disease could be prevented not only by targeting the pathogen directly (e.g. with antimicrobial or anti-adhesion agents) but also indirectly by interfering with the ecological pressure responsible for the selection of the pathogen [4]. Various methods were tried to suppress the caries activity. One of the novel strategies for prevention of dental caries is by manipulation of resident oral microorganism by ingestion of probiotic organisms [5].

The interest in probiotic therapy to prevent oral diseases has grown remarkably over the years. PROBIOTIC, meaning 'For Life' was first coined in the 1960s, by Lilly & Stillwell [6]. Food and Agriculture Organization/World Health Organization ((FAO/WHO) in 2001 defined it as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" [7].

Elimination of pathogenic members of the oral cavity can be attempted by probiotic approach because they have an ability to survive in acidic environment which will be present especially in dental caries [8]. *Lactobacilli* and *bifidobacteria* are the most commonly used and studied probiotic organisms. Replacement of cariogenic bacteria by non pathogenic bacteria improved oral health in children, which can be done with the use of probiotics [9]. Dairy foods like cheese, yoghurt and milk are considered useful vehicles

for probiotic bacteria, but an ideal administration vehicle has yet to be identified [9]. Ideally, it has been suggested that exposure early in life may facilitate a permanent installation of health promotion. Hence the probiotic vehicle should be suitable for all ages and especially for young children. In this context, ice-cream is an interesting probiotic food, as it is popular and universally liked [10].

Very few studies have been done to check the beneficial effect of probiotic organisms and limited to adults. Several studies have investigated the effect of milk containing probiotic bacteria in children and other dairy products but only two studies on ice-cream containing probiotics and its effects have been done [10]. Since very little has been explored in this part, a study was planned and carried out in children of 6-12 y age group with the aim to compare the salivary levels of *Streptococcus mutans* levels before and after consumption of probiotic and control ice-cream.

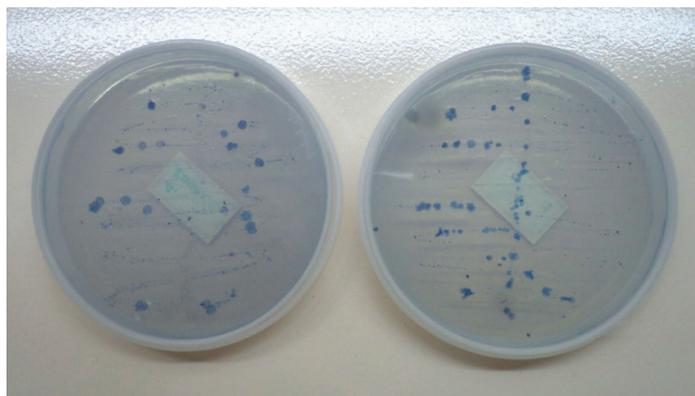
## MATERIALS AND METHODS

Prior to the study, ethical clearance was obtained on 13<sup>th</sup> Oct 2011, from the ethics committee of Navodaya Dental College and Hospital, Raichur, Karnataka, India. The study design was explained to the District educational officer of Raichur district and permission to conduct the study in urban Raichur schools was obtained. Subjects of 6 to 12 y age group were selected for this study from two different schools of Raichur city, whose parents gave full consent to participation of their child, who were residents of Raichur by birth, from same locality and have a same source of drinking water supply with fluoride content of 0.4ppm.

The children with a history of dental treatment, systemic antibiotics or topical fluoride treatments three months prior to baseline, habitual use of dairy probiotics, Xylitol chewing gums, severe medical conditions, and who are allergic to dairy products were excluded from the study. Sixty children between 6-12 y of age and with zero DMFT/dmft index based on WHO criteria [11] were selected as a sample for the study.

These children were equally divided by randomization into Group I and II. They were given proper diet instructions and were given tooth paste and brushes. Proper brushing technique was demonstrated and was instructed to maintain their oral hygiene throughout the study period.

On the next day of the oral examination 2ml of unstimulated whole saliva was collected in 5 ml disposable sterile container, between 11.00 am-12.00 pm from all the participants during the school hours. The saliva sample was transferred to the laboratory where with the help of 4 mm internal diameter inoculation loop, 10  $\mu$ l of sample was streaked on freshly prepared Mitis Salivarius Bacitracin agar culture plates for inoculation. The MSB culture plates were then incubated aerobically for 48 h. Following incubation colonies with morphological characteristics of MS were counted [Table/Fig-1]. The identification of *Streptococcus mutans* was confirmed by Gram staining method. A manual colony counter with magnifying glass was used to count the colonies and they were expressed as number of colony forming units per ml (cfu/ml) of saliva [Table/Fig-2]. By multiplying the actual colony count by  $1 \times 10^3$  semi quantification of the number of colonies was done. The readings obtained were tabulated and considered as baseline data.



[Table/Fig-1]: Culture plates with growth



[Table/Fig-2]: Manual colony counter

The study was double blind in nature. This was possible as the ice-creams, control and study had similar taste (vanilla) and were distributed in similar white cups marked 'A' as control and 'B' as study group. But the content was not known to the children or the investigator. The codes were sealed and given to the chief supervisor of the study. The sanctity of the double blind trial was strictly maintained and decoding done only after statistical evaluation of all parameters.

An equal quantity (54gms) of both probiotic and normal ice creams were given to these children for the next 7 days continuously. The probiotic ice-cream used in this study was prepared by adding a freeze dried culture of probiotic strains of *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* La-5 and manufactured by Amul India Pvt. Ltd., (Anand, Gujarat, India). The probiotic ice-cream contained  $1 \times 10^6$  CFU of each probiotic strains [10].

After the study period, on the next day the unstimulated saliva samples were collected, inoculated and the colonies were counted after incubation. Data was recorded. A washout period of 30 d was given and during this period children were instructed to follow the diet restrictions and maintain the oral hygiene as per the instructions given prior to the study. After 30 d the unstimulated saliva was collected from both the groups and again the colony counting was done.

Similarly after six months the saliva samples were collected from both the groups for colony counting. The data thus obtained were subjected to statistical analysis by using Students paired t-test and Bonferroni's correction was applied. The results were tabulated using SPSS ver 17 software.

## RESULTS

Data obtained from Baseline, after study period, after 30 days of washout period and after six months are given in [Table/Fig-3]. Findings of this study revealed that there was reduction of MS in the children who consumed probiotic ice-cream when compared to baseline which was statistically significant ( $p < 0.001$ ). Whereas in the control group there was constant increase in the MS count throughout the study period and when compared with the baseline data it was statistically non significant.

Bonferroni's correction was used in this study which is an adjustment made to P-values when several dependent or independent statistical tests are being performed simultaneously on a single data set to avoid false positive results. So in this study this correction is applied to overcome multiple comparison errors.

When the multiple comparison was made among the mean values in study group from the baseline and after seven days of study period, reduction in MS count was observed, that was statistically significant, and very highly significant when compare with baseline data and after 30 d of washout period ( $p < 0.001$ ), when compared after six months there was significance seen compared to baseline data but the average levels of *streptococcus mutans* were at the same level as after 30 d of study period [Table/Fig-4].

Whereas in control group, when comparison was made between mean values of baseline to seven days, 30 days of washout period and also after six months, there was no statistical significance found [Table/Fig-4].

The above comparisons suggested that there was significant reduction of MS in the children who consumed probiotic ice cream, effect of which reduced over the washout period and throughout the study period, there was constant increase of MS count in the control group who consumed normal ice cream.

## DISCUSSION

Advanced scientific technologies today has provided us tools to treat the infection before it causes damage. The older concept of caries being an irreversible disease is no longer accepted, as the

	group	N	Mean	Std. Deviation	t
0 Day	Group A	30	72.70	14.128	0.186 p=.853 ns
	Group B	30	73.37	13.677	
7Days	Group A	30	77.33	11.293	4.287 p<0.001 vhs
	Group B	30	63.87	12.982	
30 Days	Group A	30	79.90	11.183	6.368 p<0.001 vhs
	Group B	30	60.03	12.920	
6 Months	Group A	30	72.80	13.828	0.550 p=.584 ns
	Group B	30	74.70	12.909	

**[Table/Fig-3]:** Average streptococcus mutans counts at baseline, 7 days, 30 days and after 6 months  
 p<0.001 - Significance; N = Number of subjects; t- Students paired; t test value; ns = Not significant; vhs = very highly significant; Group A- control; Group B - intervention

Group	(I) time	(J) time	Mean Difference (I-J)	p
Group A N=30	Base	7 days	-4.633	.959
		30 days	-7.200	.179
		6 months	-.100	1.000
	7 days	30 days	-2.567	1.000
		6 months	4.533	1.000
	30 days	7 days	-2.567	1.000
30 days	6 months	7.100	.193	
Group B N=30	Base	7 days	9.500	0.036 sig
		30 days	13.333	<0.001 vhs
		6 months	-1.333	1.000
	7 days	30 days	3.833	1.000
		6 months	-10.833	0.011 ns
	30 days	7 days	3.833	1.000
30 days	6 months	-14.667	<0.001 vhs	

**[Table/Fig-4]:** Multiple Comparisons of the groups  
 p<0.001 - Significance; N = Number of subjects; ns = not significant; vhs= very highly significant

initial demineralization of tooth structure can be diagnosed with the newer diagnostic equipments and the tooth can be remineralized with diet control, antimicrobial mouthwashes, fluoride supplements and calcium-phosphate remineralizing agents [12].

India, a developing nation, has shown an inclined trend of caries in children over a relatively short period of time. In 1940, the prevalence of dental caries in 6 to 12-year-old school children in India was 55.5% and it rose to 68% in the 1960s and climbed to 89% in subsequent years [13-15]. The age group was chosen in this study was based on these inferences.

To avoid the comparative bias, children with zero DMFT/dmft [10] were chosen as the person with the caries lesions in the mouth will have high levels of *Streptococcus mutans* in the saliva. To avoid subjective bias, children were selected are from the same locality by birth and have a common source of drinking water supply. The children were given proper diet charts and were instructed not to consume any kind of probiotic containing products or fluoride supplements. Triclosan containing toothpaste has been demonstrated to be highly effective in plaque and gingivitis control [16]. Hence, each child was given a tooth brush and paste containing 1000ppm of fluoride (Colgate® Total) one month prior to the study and were trained to brush using proper brushing technique and were instructed to continue to use throughout the study period, which helped in maintaining the proper and uniform oral hygiene in the children.

A new promising approach to prevent dental caries is studied by upsetting the cariogenic bio film by the use of Probiotics also

referred to as Replacement therapy. Replacement therapy involves the use of a harmless effector strain that is permanently colonized in the host's micro flora [8].

Probiotics create a biofilm which acts as a protective lining for tissues and keep pathogens away, with no host environment for pathogens [5]. Studies done on probiotic approach were first to the alteration of the gut flora and were successful. Similarly it was tried to alter the microbial flora of the oral cavity, in reducing the oral diseases especially caries and found successful mainly in reducing the levels of *Streptococcus mutans* species [12]. When probiotics were added while oral biofilm formation, Lactobacillus species strongly inhibited the growth of the *Streptococcus mutans* [17].

The main probiotic preparations currently on the market are the lactic acid-producing bacteria are *Bifidobacterium* and *Lactobacillus* [8]. Dairy products containing *Lactobacillus reuteri* have been on the market. Besides, *Lactobacillus rhamnosus* is safe and useful in its intended uses as a functional food or ingredient its storage stability in refrigerated milk-based foods was proved excellent [12].

In daily routines, administration of probiotics to children is difficult. A previous study with a pacifier with slow releasing probiotic lozenges was also tried [18]. Various other vehicles have also been tried like tooth pastes, tablets, drinks, syrups, and dairy products. One of the dairy products in which probiotic organisms can be added and supplied is ice creams.

A study was done in Croatian population of 50 participants who were given toothpaste containing probiotics. After four weeks of usage there was significant reduction of *streptococcus mutans* in the participants who has high caries index before the commencement of study [19]. Comparative study of Kefir yogurt drink and sodium fluoride mouth rinse on SMS showed that dairy products as well as fluoride can reduce the levels of *streptococcus mutans* and concluded that dairy products containing probiotics can be used [20]. The effect of tablets containing probiotic *lactobacilli* were checked on the early carious lesions in adolescents using quantitative light induced fluorescence (QLF) after three months of tablet ingestion showed no significant difference [21].

Ice-cream is a delicious, wholesome, nutritious frozen dairy product, which is widely consumed in different parts of the world by all age groups and especially children. Even though ice-cream has sweetening and flavouring agents, ice-cream has nutritional significance and could be an interesting carrier of probiotic food, with reported advantages [22]. Dairy products like milk and yogurt may not be liked by all the children and there are chances that children may not agree to continue with the study but ice-cream is universally accepted by children compared any other dairy products. Hence, Ice-cream was used as a vehicle in this study based on above mentioned reasons and it was well accepted by the participants.

In our study, after seven days of ice-cream consumption there was very highly significant reduction in the salivary levels of MS in children of study group when compared to the baseline (p<0.001). Which are in accordance with the previous studies on which used ice-cream as vehicle [10,23]. Whereas in the control group there was increase of salivary MS level compared to baseline.

In a short term clinical study were children were given probiotic containing ice-cream and curd and checked for the immediate effect and also after a week's period showed significant reduction in the salivary *streptococcus mutans* levels than in control group. Which supports this study [24].

A washout period of 30 d was given and salivary levels of MS were again calculated. When the comparison was made, there was very highly significant (p<0.001) reduction of salivary MS in the study group even after the washout period when compared to baseline data. But there was no significant reduction seen when compared after the study period and at the end of washout period. This

inferred that the synergistic effect of probiotic strains reduced over the washout period. In contraire there was overall increase in the *Streptococcus mutans* levels in the control group throughout the study period, findings of which were statistically nonsignificant.

The study was continued for six more months to check the synergistic effects of probiotic organisms on the salivary *Streptococcus mutans* levels. But comparisons to the baseline data and levels of *streptococcus mutans* after six months showed that there was significance in study group because levels remained constant but in control group there was no significance. This shows in this study, there was no synergistic effect of probiotic organisms for as long as six months.

Various authors confirmed that the probiotic organisms are only transiently harboured in mouth and upon discontinuation, they disappear rapidly [25-27]. A recent systematic review and meta analysis on probiotics and its effect on *Streptococcus mutans* concluded that there was significant reduction in the *Streptococcus mutans* in probiotic group but even after probiotics use there was no significant effects on *lactobacilli* counts. Study concluded saying probiotics definitely has effect on *streptococcus mutans* and can be used as caries preventive method [28].

In this respect, our findings that the salivary MS levels were insignificantly lower at the end of washout period and after 6 months when compared after the study period, may indicate that, for long term reduction of salivary MS level, the ingestion of probiotic organisms should be given for longer intervals. Which is also confirmed by the studies done where ice-cream was used as a vehicle [10,23,24]. This clearly illustrates the fact that the optimal dose needed for bacterial suppression in the oral environment for long duration is still to be determined.

## CONCLUSION

Use of probiotic organisms for restoring oral health and prevention of caries is gaining interest in the researchers. Many studies conducted with probiotic strains originally suggested for gut health were successful. Although the mechanism of action are unclear, various short term studies using different probiotic containing products especially dairy products including ours, concluded that there is definitive effect of probiotic organisms in reducing the salivary levels of *Streptococcus mutans* for short time. Further studies on the long term or synergetic effect of the probiotic organisms on the caries causative bacteria, oral health and optimum dosage of the probiotic organisms are still need to be explored.

## ACKNOWLEDGEMENT

Mrs. Kamala Navaratna Msc., Professor & Head, Department of Microbiology, L.V.D. college of Arts & science, Raichur for her co operation, valuable guidance and help in our study.

## REFERENCES

- [1] Petersen PE, Bougreois D, Ogawa H, Estupian-Day S, Ndiaye CH. The global burden of oral diseases and risks to oral health. *Bull World Health Organ.* 2005;83:661-69.
- [2] Berg JH. Early dental caries detection as a part of oral Health maintenance in young children. *Compendium.* 2005;26:24-29.

- [3] Loesche WJ. Role of *Streptococcus mutans* in human dental decay. *Microbiological reviews.* 1986;35:3-80.
- [4] Philip D Marsh. Dental plaque as a biofilm and a microbial community—implications for health and disease. *BMC Oral Health.* 2006;6(Suppl 1):S14.
- [5] Meurman JH, Stamatova I. Probiotics: contributions to oral health. *Oral Dis.* 2007; 13:443-51.
- [6] Lilly DM, Stillwell RH. Probiotics. Growth promoting factors produced by microorganisms. *Science.* 1965; 147:747-48.
- [7] Report of a Joint FAO/WHO Expert Consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. 1–4 October; Cordoba; Argentina; 2001; Available on internet, available on ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf.
- [8] Meurman JH. Probiotics: do they have a role in oral medicine and dentistry? *Eur J Oral Sci.* 2005;113:188-96.
- [9] Sameer A. The synergism of Probiotics in dentistry. *Saudi dental journal.* 2007;17:3.
- [10] Singh RP, Damle SG, Chawla A. salivary *streptococcus mutans* and *lactobacilli* modulations in young children o consumption of probiotic ice-cream containing *Bifidobacterium lactis* Bb12 and *Lactobacillus acidophilus* La5. *Acta Odontol Scand.* 2011; Early Online, 1–6.
- [11] World Health Organization. Oral Health Surveys Basic Methods. 4<sup>th</sup> ed. Geneva: World Health Organization; 1997. pp. 40–44.
- [12] Ranganathan R, Vaidya R. Preventing dental caries the probiotic approach. *The Journal of Ahmadabad Dental College and Hospital.* 2011;2:60-65.
- [13] Ditmyer M, Dounis G, Mobley C, Schwarz E. Inequalities of caries experience in Nevada youth expressed by DMFT index vs. Significant Caries Index (SiC) over time. *BMC Oral Health.* 2011;11:12.
- [14] Joshi N, Rajesh R, Sunitha M. Prevalence of dental caries among school children in Kulasekharam village: a correlated prevalence survey. *J Indian Soc Pedod Prev Dent.* 2005;23(3):138-40.
- [15] Damle SG, Patel AR. Caries prevalence and treatment needs amongst children at Dharavi, Mumbai. *Community Dent Oral Epidemiol.* 1994;22:62-63.
- [16] Vered Y, Zini A, Mann J, DeVizio W, Stewart B, Zhang YP, Garcia L. Comparison of a dentifrice containing 0.243% sodium fluoride, 0.3% triclosan, and 2.0% copolymer in a silica base, and a dentifrice containing 0.243% sodium fluoride in a silica base: a three-year clinical trial of root caries and dental crowns among adults. *J Clin Dent.* 2009;20:62-65.
- [17] Lee SH, Kim YJ. A comparative study of the effect of probiotics on cariogenic biofilm model for preventing dental caries. *Arch Microbiol.* 2014;196:601-09.
- [18] Çağlar E, kusu OO, Cildir SK, Kuvvetli SS, Sandalli N. A probiotic lozenge administered medical device and its effect on salivary mutans streptococci and *lactobacilli*. *Int J Paediatr Dent.* 2008;18:35-39.
- [19] Majstorovi, M, Vrani, DN, Szivovicza L. Recent achievements in preventive dentistry by introducing new probiotic toothpaste. *Coll Antropol.* 2013; 37:1307-12.
- [20] Ghasempour M, Sefidgar SA, Moghadamnia AA, Ghadimi R, Gharekhani S, Shirkhani L. Comparative study of Kefir yogurt drink and sodium fluoride mouth rinse on salivary mutans streptococci. *J Contemp Dent Pract.* 2014;15:214-17.
- [21] Keller MK, Nøhr Larsen I, Karlsson I, Twetman S. Effect of tablets containing probiotic bacteria (*Lactobacillus reuteri*) on early caries lesions in adolescents: a pilot study. *Benef Microbes.* 2014;5:403-07.
- [22] Sharareh H, McMahon DJ. Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice-cream for use as a probiotic food. *J Dairy Sci.* 1992;75:1415-22.
- [23] Çağlar E, Kusu OO, Kuvvetli SS, Cildir SK, Sandalli N, Twetman S. Short-term effect of ice-cream containing *Bifidobacterium lactis* Bb-12 on the number of salivary mutans streptococci and *lactobacilli*. *Acta Odontol Scand.* 2008;66: 154–58.
- [24] Chinnappa A, Konde H, Konde S, Raj S, Beena JP. Probiotics for future caries control: a short term clinical study. *Indian J Dent Res.* 2013;24:547-49.
- [25] Yli-Knuuttila H, Snäll J, Kari K, Meurman JH. Colonization of *Lactobacillus rhamnosus* GG in the oral cavity. *Oral Microbiol Immunol.* 2006;21:129–31.
- [26] Näse L, Hatakka K, Savilahti E, Saxelin M, Pönkä A, Poussa T, et al. Effect of long term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. *Caries Res.* 2001;35:412–20.
- [27] Nikawa H, Makihira S, Fukushima H, Nishimura H, Ozaki Y, Ishida K. *Lactobacillus reuteri* in bovine milk fermented decreases the oral carriage of mutans streptococci. *Int J Food Microbiol.* 2004;95:219–23.
- [28] Laleman I, Detaillieu V, Slot DE, Slomka V, Quirynen M, Teughels W. Probiotics reduce mutans streptococci counts in humans: a systematic review and Meta analysis. *Clin Oral Investig.* 2014; 18:1539-52.

### PARTICULARS OF CONTRIBUTORS:

1. Senior Lecturer, Department of Pediatric and Preventive Dentistry, Kannur Dental College, Anjarakandy, Kannur, Kerala, India.
2. Professor & Head, Department of Pediatric and Preventive Dentistry, Navodaya Dental College, Raichur, Karnataka, India.
3. Professor, Department of Pediatric and Preventive Dentistry, Navodaya Dental College, Raichur, Karnataka State, India.
4. Reader, Department of Pediatric and Preventive Dentistry, Navodaya Dental College, Raichur, Karnataka State, India.
5. Reader, PMNM Dental College & Hospital, Bagalkot, Karnataka, India.
6. Senior Lecturer, Department Periodontics, Kannur Dental College, Anjarakandy, Kannur, Kerala, India.

### NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Devasya Ashwin,  
8/491 Krishna Nagar, Kumbala, Kerala-671321, India.  
E-mail: ashwindkumbala@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Aug 24, 2014**  
Date of Peer Review: **Nov 21, 2014**  
Date of Acceptance: **Dec 03, 2014**  
Date of Publishing: **Feb 01, 2015**