Malaysian Dental Journal (2015) 37(1) 1-8

MALAYSIAN DENTAL JOURNAL

Evaluation of Clinical Efficacy of Two Chemo-Mechanical Caries Removal Agents in Primary Molars – A Comparative Study

Venkatesh Babu NS[1], Basavaraj N[2], Milind LS[2], Golai S[3]

[1] *Professor and Head, Department of Pedodontics and Preventive Dentistry, V.S. Dental College and Hospital, Karnataka, India*

[2] *Formerly Postgraduate Student, Department of Pedodontics and Preventive Dentistry, V.S. Dental College and Hospital, Karnataka, India*

[3] *Assistant Professor, Department of Pedodontics and Preventive Dentistry, Chitwan Medical College and Teaching Hospital Bharatpur – 10, Chitwan, Nepal*

ABSTRACT

Background: In children, caries removal by means of conventional rotary instruments is often associated with pain and discomfort. An alternative method to conventional caries removal by means of rotary instruments is the use of chemo-mechanical caries removal agents. *Aim*: To evaluate and compare the efficacy of two chemo-mechanical caries removal agents Carisolv and Papacarie in reducing cariogenic flora in primary molars employing bacteriological evaluation. *Materials and Methods*: Forty children aged 3-9 years were randomly divided into Carisolv and Papacarie group. In each group, caries sample was taken with a sterile instrument under rubber dam isolation before and after the application of the respective chemo-mechanical caries removal agents. The samples were serially diluted and plated onto two different agar plates, Schaedler agar to determine the total viable count of bacteria and MRS agar to determine the viable count of Lactobacilli. The agar plates were incubated for 3 days (Schaedler agar at 37^{o}C , MRS agar at 32^{o}C). The number of colonies formed were determined and expressed as CFU (colony forming units) per sample. *Results*: The difference in mean reduction of CFU's between Carisolv group & Papacarie group was found to be 77.03. Higher mean reduction of microbial counts was observed in the Papacarie group (157.98) compared to Carisolv group (80.95). This difference in mean reduction between the two groups was found to be statistically significant ($p< 0.01$). *Conclusion*: Papacarie can be an effective clinical alternative to Carisolv for the removal of dentinal caries in cavitated primary molars.

Key Words: Carisolv, Papacarie, Colony forming units, Viable bacterial count, Primary molars

Please cite this article as: Venkatesh Babu NS, *et al*. Evaluation of clinical efficacy of two chemo-mechanical caries removal agents in primary molars – a comparative study. Malaysian Dental Journal 2015; 37(1): 1-8.

INTRODUCTION

Dental caries is a complex microbial disease that affects a majority population of the world, regardless of age, gender and ethnicity. Dental caries is a localized destruction of tooth tissue by specific dental plaque bacteria that ferment dietary sugar to organic acids. It is one of the most common oral diseases in children and adolescents.

In children, caries excavation by means of conventional rotary instruments like micromotor and air rotor is often associated with pain and discomfort. The main disadvantages of the traditional rotary drills are, the perception of patients that drilling is unpleasant, local anesthesia is frequently required, drilling can cause harmful thermal and pressure effects on the pulp, the use of hand piece may result in removal of normal, uninfected dentin, resulting in excessive loss of sound tooth tissue.[1,2]

The studies on chemo-mechanical caries removal were first carried out by Habib et al.[3] using 5% sodium hypochlorite solution, which is a proteolytic agent. But sodium hypochlorite was found eventually too corrosive to be used in healthy tissue, Goldman et al. (1976) made an attempt to minimize the problem by introducing GK-101 for removal of dental caries.[4] In 1984, Food and Drug

Authority (FDA) approved its use in United States of America (USA) and it was marketed as Caridex system in 1985.[5] Despite its effectiveness, Caridex had certain disadvantages such as long working time, less shelf life and required a large volume of solutions along with a special pump.[6] Rolf Bornstein et al. in mid-90's introduced Carisolv as a successor to Caridex.[7] Carisolv was quite a success in the field of dentistry but with its long term use certain drawbacks of the system have been reported. One of the drawbacks is requirement of customized instruments that increased the cost of the product.

In 2003, a research project in Brazil conducted by Bassadori et al.[8] led to the development of a new agent to simplify the use of the chemo-mechanical method for caries removal, which was made available for use in public health in 2005 as Papacarie. Papain gel, the basic component of this product, which has bacteriostatic, bactericidal and anti-inflammatory characteristics.

The present study was undertaken to determine the efficacy of two chemo-mechanical caries removal agents - Carisolv and Papacarie for caries excavation from primary molars using bacteriological evaluation. The total viable bacterial counts were determined from the infected dentine before the use of chemo-mechanical agents and from the top layers of the residual dentine after caries removal by Carisolv and Papacarie. A comparison of the reduction in microbial counts achieved by the use of two agents was carried out.

MATERIALS AND METHODS

The present in-vivo study was carried out involving 40 healthy children aged between three to nine years. In each child, one carious primary molar fulfilling the selection criteria was included in the study. Teeth were selected according to the following criteria which include open carious lesions with dentin involvement, but not involving pulp, the access of the carious lesion has to be large enough to allow the penetration of the excavator, absence of proximal carious lesions, asymptomatic vital teeth, without clinical or radiological evidence of pulp, furcation or periapical pathoses, and absence of any developmental defects. Only cooperative children as judged by the Frankle behavior rating scale were selected.[9] A dental examination was carried out for all children and the degree of carious involvement of primary molars were determined with dental probe, using visual and tactile criteria. Informed consent was obtained from parents/guardians of each child for participation in the study. Institutional ethical committee approval was also obtained.

Children were randomly selected to be divided into two groups of 20 each according to the chemo-mechanical caries removal agent used for caries removal. Group I represented the children teeth treated with Carisolv agent and Group II represented the children teeth treated with Papacarie.

The initial samples from the superficial carious lesion were taken under rubber dam isolation before the use of chemo-mechanical agents and from the top layers of the residual dentine after removal of caries by the respective gels with the help of a sterile spoon excavator. The samples were then transferred into sterile vials that contain 500µL of sterile isotonic saline for microbiological analysis (Figure 1, 2).

Figure 1. Collection of sample for microbiological evaluation.

Figure 2. Collected samples in sterile vials with screw caps.

Caries removal by Carisolv

Carisolv system is marketed as multi-mix package with two syringes. According to manufacturer's instructions, the multi-mix package was removed from the refrigerator approximately one hour before treatment. Carisolv gel was mixed using the multi-mix syringe dispenser. The gel components are extruded when the plunger is pressed. The gel components mixed automatically in the correct proportions in the tip (static mixer) of the syringe and dispensed into a dappen glass and then applied onto the dentinal carious lesion using a sterile plastic filling instrument. The mixed gel need to be used immediately, as its effectiveness begins to deteriorate after 20 minutes. The lesion was completely covered by the gel for 30 seconds. On application, the gel was clear, but became opaque or cloudy when it was contaminated with the debris during caries removal. Following this, the softened dentine was gently excavated using Star 3 double ended excavator [Mediteam Dental AB, Sweden] as per manufacturer's instructions. The softened carious dentine was scraped gently without any cutting motion so that it does not promote any kind of stimulus or pressure to the tooth. The material was re-applied if necessary till complete removal of caries was achieved. The gel was then removed and the cavity was wiped with a moistened cotton pellet, rinsed and air dried.

Caries removal by Papacarie

Papacarie is supplied as a single component in a syringe. According to manufacturer's instructions, it was applied to the dentinal carious lesion using a sterile plastic filling instrument. The lesion was completely covered by the gel for 30 seconds. Softened carious dentin was gently scraped with spoon excavator without applying pressure in a pendulum movement. The gel was re-applied till the cavity appeared vitreous, which indicated that the cavity was free of caries. The gel was then removed and the cavity was wiped with a moistened cotton pellet, rinsed and air dried.

In both groups, the complete removal of caries was evaluated by visual and tactile examination.[10] After caries excavation teeth were restored with Type-2 Glass ionomer cement (GC Corporation, Japan).

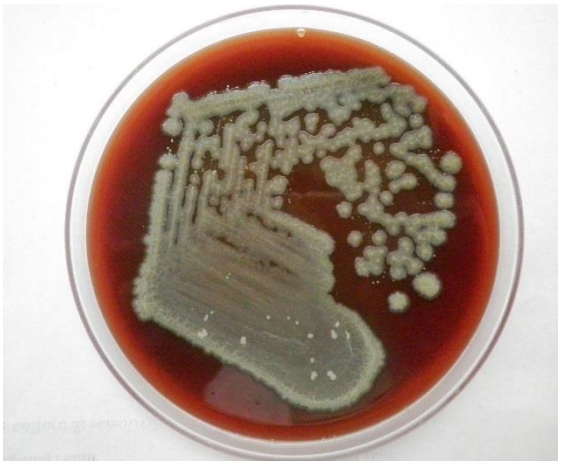

Figure 3. Growth on Schaedler agar plate – pretreatment.

Figure 4. Growth on Schaedler agar plate – post treatement.

Microbial Cultivation and Evaluation

The collected dentin samples were immediately transferred into sterile vials with screw caps and processed in the laboratory within two hours. Each sample was mixed with 500µL sterile isotonic saline and vortexed for about 15 seconds in order to dislodge the bacteria from dentine. The samples were then serially diluted and plated onto two different agar plates. Schaedler agar (Hi Media, Mumbai , India) was used to determine the total viable bacterial counts and MRS agar (Hi Media, Mumbai , India) was used to determine the viable counts of Lactobacilli (Figure 3, 5).

Figure 5. Growth on MRS agar plate – pretreatment.

The agar plates were incubated for 3 days (Schaedler agar at 37°C and MRS agar at 32°C). The number of colonies formed were determined and expressed as CFU (colony forming unit) per sample (Figure 4, 6). Samples from colony growth from MRS agar plates were examined under light microscope to determine the morphological types of the bacteria. It was observed that the bacteria were of regular rod-shape in all cases, which is typical of lactobacilli.

Total viable bacterial count and viable count of lactobacilli in dentine samples of both the groups before and after application of respective gels were compared and statistically analyzed.

RESULTS

The present in-vivo study was conducted to determine the efficacy of two chemo-mechanical caries removal agents Carisolv and Papacarie in primary molars employing bacteriological evaluation.

Comparison of reduction in the total viable bacterial count and viable count of lactobacilli in the Carisolv group.

The mean reduction in total viable bacterial count as observed on Schaedler agar plate was found to be 59.8 CFU. The mean reduction in viable count of lactobacilli as observed on MRS agar plate was found to be 102.1 CFU. Reductions on both agar plates were found to be statistically significant ($p<0.01$) (Table 1).

Figure 6. Growth on MRS agar plate – post treatment.

Comparison of reduction in the total viable bacterial count and viable count of lactobacilli in the Papacarie group.

The mean reduction in total viable bacterial count as observed on Schaedler agar plate was found to be 201.0 CFU. The mean reduction in viable count of lactobacilli as observed on MRS agar plate was found to be 114.95 CFU. Reductions on both agar plates were found to be statistically significant ($p<0.01$) (Table 2).

Comparison of reduction in the bacterial counts between both the groups.

The difference in mean reduction of CFU between thePapacarie group &Carisolv group was found to be 77.025. Higher mean reduction of microbial counts was observed in Papacarie group compared to Carisolv group. This difference in the mean reduction between the two groups was found to be statistically significant ($p<0.01$) (Table 3).

DISCUSSION

Dental caries is considered to be one of the most serious dental diseases that result in localized dissolution of organic and destruction of inorganic tissues of the tooth. Neglecting the treatment could endanger the tooth pulp resulting in subsequent pain and tooth loss. In 1893, GV Black proposed his principle “extension for prevention” in the operative treatment of carious lesions. The fact is that the Black’s principle was constrained by both the knowledge of disease process and restorative materials presented at that time.[11] However, the

Table 1. Comparison of CFU in Group I (Carisolv)

Agar Plate	*CFU*	*Mean*	*Std dev*	*SE of Mean*	*Mean difference*	*Z*	*P-Value*
Schaedler	Before	264.50	156.36	34.96	196.00	-3.924	<0.001*
	After	68.50	62.26	13.92			
MRS	Before	157.00	156.30	34.95	118.85	-3.921	<0.001*
	After	38.15	36.72	8.21			

*denotes significant difference

Table 2. Comparison of CFU in Group II (Papacarie)

Agar Plate	*CFU*	*Mean*	*Std dev*	*SE of Mean*	*Mean difference*	*Z*	*P-Value*
Schaedler	Before	89.00	78.60	17.57	57.95	-3.926	<0.001*
	After	31.05	30.87	6.90			
MRS	Before	154.50	134.62	30.10	102.10	-3.924	<0.001*
	After	52.40	47.58	10.64			

*denotes significant difference

Table 3. Comparison of the reduction in CFU between the two groups (Mann-Whitney test)

Group	*Mean*	*Std dev*	*SE of Mean*	*Mean difference*	*Z*	*P-Value*
Group I	80.65	77.82	12.30	77.025	-2.669	0.004*
Group II	157.43	118.68	18.76			

*denotes significant difference

demand of removing sound enamel and dentin has been dramatically changed over the years as a result of advancement in the adhesive restoratives and the alternative approaches of both caries removal and cavity preparation.[12,13,14] In the last few decades, a growing interest has been noticed to develop towards alternative minimally invasive techniques like air abrasion, lasers, ultrasonic, ozone therapy and others. It is obvious that most of the mentioned techniques are trying to achieve a conservative approach for removing dental caries. This issue stimulated the development of a clinical approach, known as atraumatic restorative treatment (ART) that involves excavation and removal of the gross caries with just hand instruments and subsequently restoring the cavities with adhesive restorative material with bacteriostatic effect.[15]

Goldberg and Keil in 1989[16] discovered the efficacy of the collagenase enzyme, in excavating infected and soft carious dentine two to five hours after its application. This resulted in a residual sound layer of dentin with no bacteria within the exposed collagen of the dentinal floor. Another enzyme named Pronase was discovered for the same purpose. In spite of the potential effectiveness, the slow action of this mode for caries excavation limits its clinical use.[16,17] Combination of the mechanical means of caries removal using hand instruments and use of chemical solvents resulted in the development of a new method of caries removal called as the chemo-mechanical caries removal method. Although, the chemo-mechanical caries removal approach has been suggested a long time ago, the interest regarding this method has recently increased. This innovative method appears to be efficient in removing soft, infected dentine without altering the healthy dental tissue. The present study was performed to assess the efficacy of this new approach to remove carious lesion in primary teeth. The micro flora is one of the main causative factors in caries initiation and progression, therefore, it is essential to reduce the microbial counts in carious lesions. One of the requirements for a chemo-mechanical agent used for caries removal include the capability to further degrade partially destroyed collagen. It is thought that the deep

layers of the dentine are preserved and only the infected layers will be removed. Habib and Kronman et al[3] studied the effect of a non-specific proteolytic agent, sodium hypochlorite (NaOCl) on infected dentine. This was shown to dissolve not only necrotic organic material, but also affected sound dentine. In order to reduce this aggressive effect, the Carisolv system was developed by the Swedish Medi-Team in 1997. This system is marketed as multi-mix package with two syringes. One syringe contains 0.5% sodium hypochlorite and the other syringe containing gel of three amino acids glutamic acid, leucin and lysine. Carboxyl methyl cellulose is added to make the gel viscous. Components of these two syringes have different charges which help the electrostatic attraction of either the hydrophilic (positively or negatively charged) or hydrophobic (none charged) patches that normally form the peptide chains of all proteins including collagen[18] On the other hand, Papacarie (formulae Aeao, Sao Paulo, Brazil) was introduced in Brazil in 2003 as a new chemo-mechanical caries removal agent. It is available as aqua blue coloured gel in a single syringe (3ml). It consists of papain, chloramines, toluidine blue dye, water, salts and thickeners. Papain is a proteolytic enzyme. It has bactericidal, bacteriostatic and anti-inflammatory characteristics. Similar to the human pepsin, papain acts as a debridingand anti-inflammatory agent which does not damage the healthy tissue and accelerates the cicatrical process. Flindt M (1979) demonstrated that papain acts only in infected tissues because of absence of the plasmatic anti-protease called alpha-1 anti-trypsin.[19] However, alpha-1 anti-trypsin which is present in sound tissues, therefore it inhibits protein digestion. The absence of the alpha-1 anti-trypsin in infected tissues allows papain to break the partially degraded molecules. Toluidine blue is an antimicrobial agent and water acts as a carrier medium. Thus with the help of each of its constituents, Papacarie achieves synergistic action that facilitates caries removal and increased antimicrobial properties.

Dawkins et al.[20] showed that papain also has bactericidal and bacteriostatic properties which inhibit the growth of gram positive and gram negative microbes. Papacarie also contains chloramines, salts and thickening agent in addition to papain. Chloramine has bactericidal and disinfectant properties. The antiseptic properties of chloramine were recently documented in an in-vitro study.[21] According to Maragakis et al.,[22] the partially degraded collagen in carious dentine was chlorinated by chemo-mechanical caries removal solutions. The chlorination affects the secondary or quaternary structure of collagen, by disintegrating hydrogen bonding and thus enabling the carious tissue removal.

An in vitro evaluation of cytotoxicity of papacarie using various concentrations of papain (2, 4, 6, 8, and 10%) on fibroblast culture found no cytotoxic effects, suggesting that Papacarie is safe and harmless to use in children.[23] The present study showed significant reduction of mean total viable bacterial count which is in accordance with the findings of studies conducted by Kneist and Heinrich-Wetzein,[23] Azrak et al.[12] and Subramaniam et al.[24]

Kidd et al. have reported that there is an association between the number of microorganisms present and the consistency of the carious dentine.[25] They also found that the number of microorganisms isolated from a site is also influenced by the method of sample taking. In the present study, the dentine samples were carefully removed with a sterile excavator. This method was chosen to reduce the risk of accidental pulpal exposure, especially when sampling hard dentine.

Singh et al compared the antimicrobial efficacy, in terms of time consumption and pain perception of chemo-mechanical caries removal agent Papacarie and conventional method of caries removal in a rural population in India. They concluded that Papacarie was effective for the removal of occlusal dentinal caries in cavitated primary molars.[26]

Bohari et al evaluated four different techniques of caries excavation in primary teeth namely, air rotor, Carisolv, Papacarie and Er:YAG laser. Results showed that laser irradiation and chemo-mechanical caries removal methods are comparable to conventional approaches in terms of effectiveness and are less painful.[27]

Mutans streptococci are mainly implicated with the initiation of enamel caries, and are rarely the predominant species isolated from carious dentine. The composition of the carious microflora

becomes more complex as the lesions progress and mainly Gram-positive rods belonging to the genera Propionibacterium, Eubacterium, Rothia, Lactobacillus, Bifidobacterium and Actinomyces, become the predominant cultivable organisms in teeth without endodontic infections.[28] Our study found lactobacilli growth on MRS agar plate in all the samples (100%). Similar finding was reported by Nancy J and Dorignac G (1992).[29]

In this study, comparison of the two chemo-mechanical caries removal agents from a bacteriological point of view showed that the total microbial count was reduced to less than 102 CFU in 95.4% of the samples after treatment with Papacarie and in 90% of the samples after the application of Carisolv. The viable count of the lactobacilli was also reduced to less than 102 CFU in 94% of the samples after treatment with Papacarie and in 89.2% of the samples after treatment with Carisolv. This low number of microbial counts is considered to be clinically acceptable by several authors.[25,30,31] The total viable bacterial count and viable count of lactobacilli, after caries excavation by respective chemo-mechanical agents was found to be less than 102 CFU, which is in accordance with findings of Kidd et al.[30] In the present study, Papacarie has shown superior results in the reduction of microbial counts in the dentinal samples as compared to Carisolv. Thus, Papacarie can be an effective clinical alternative to Carisolv for the removal of dentinal caries in cavitated primary molars.

REFERENCES

1. Banerjee A, Watson TF, Kidd EA. Dentine caries excavation: a review of current clinical techniques. Br Dent J. 2000;188:476-82.
2. Willershausen B, Azrak B, Wilms S. Fear of dental treatment and its possible effects on oral health. Eur J Med Res. 1999;4:72-7.
3. Habib CM, Kronman J, Goldman M. A chemical evaluation of collagen andhydroxyproline after treatment with GK-101 (N-chloroglycine). PharmacolTher Dent. 1975;2:209-15.
4. Goldman M, Kronam JH. A preliminary report on a chemo mechanical meansof caries removal. J Am Dent Assoc. 1976;93:1149-53.
5. Schutzbank SG, Galaini J, Kronman JH, Goldman M, Clarke RE. A comparative in vitro study of the effect of GK-101 and GK-101E in cariesremoval. J Dent Res. 1978;57:861-4.
6. Beeley JA, Yip HK, Stevenson AG. Chemo-mechanical caries removal: a review of techniques and latest developments. Br Dent J. 2000;188:427-30.
7. Wedenberg C, Bornstein R. Pulpal reactions in rat incisors to Caridex. Aust Dent J. 1990 Dec;35(6):505-8..
8. Bussadori SK, Castro. LC, Galvao AC. Papain gel: a new chemo¬mechanicalcaries removal agent. J Clin Pediatr Dent. 2005;30:115-19.
9. Frankle SN, Shiere FR, Fogel HR. Should the parent remains with the childin the dental operatory. J Dent Child. 1962;29:150-4.
10. Lozano-Chourio MA, Zambrano O, González H, Quero M. Clinical randomized controlled trial ofchemo mechanical caries removal (Carisolv). Int J Pediatr Dent 2006;16:161-7.
11. Braun A, Christian S, Frentzen M. Chemo-mechanical caries removal (Carisolv) in comparison to conventional methods of caries excavation. Caries Res 2001;35:310.
12. Azrak B, Callaway A, Grundheber A, Stender E, Willershausen B. Comparison of the efficacy of chemo mechanicalcariesremoval (Carisolv) with that of conventional excavation in reducing the cariogenic flora. Int J Paediatr Dent 2004;14:182–191.
13. Yazici, Atilla P, Ozgunaltay G, Muftuoglu S. In vitro comparison of the efficacy of Carisolv and conventional rotary instrument in caries removal. J Oral Rehabil 2003;30:1177-82.
14. Fluckiger, Waltimo T, Stish T, Lussi A. Comparison of chemo mechanical caries removal using Carisolv or conventional hand excavation in deciduous teeth in vitro. J Dent 2005;33:87-90.
15. Ivar A, Valeria V. A review of atraumatic restorative treatment. Int Dent J 1999;49:127-31.
16. Goldberg M, Keil B. Action of a bacterial Achromobacter collagenase on the soft carious dentin: an in vitro study with the scanning electron microscope. (abstract). J BiolBuccale 1989;17:269-74.

17. Nordbo H, Brown G, Tjan AH. Chemical treatment of cavity walls following manual excavation of carious dentin. Am J Dent 1996;9:67-71.
18. Ericson D. The efficacy of a new gel for chemo mechanical caries removal. (Abstract) J Dent Res. 1998;77:1252.
19. Flindt M. Health and safety aspects of working with enzymes. ProcessBiochem. 1979;13:3-7.
20. Dawkins G, Hewitt H, Wint Y, Obiefuna PH, Wint B. Antimicrobial effects of Carica papaya fruit on common wound organisms. West Indian Med J 2003;52:290-2.
21. Fuursted K, Hjort A, Knudsen L. Evaluation of bactericidal activity and lag ofregrowth (post antibiotic effect) of five antiseptics on nine bacterial pathogens. Antimicrob Chemother. 1997;40:221-6.
22. Margakis GM, Hahn P, Hellwig E. Clinical evaluation by patients of chemo mechanical varies removal in primary molars and its acceptance by patients. Caries Res. 2001;35:205-10.
23. Kneist S, Heinrich-Weltzien R: Antibacterial action of Carisolv. In: AlbrektssonT, Bratthall D, Glantz P, Lindhe J, editors). Tissue preservation in cariestreatment. London: Quintessence; 2001. p.205-19.
24. Subramaniam P, Babu KL, Neeraja G. Comparison of the antimicrobialefficacy of chemo mechanical caries removal (Carisolv) with that ofconventional drilling in reducing cariogenic flora. J Clin Pediatr Dent. 2008;32:215-9.
25. Kidd EAM, Joyston-Bechal S, Beighton D. Microbiological validation of assessments of caries activity during cavity preparation. Caries Res 1993;27:402–8.
26. Sanjeet Singh, Deepti Jawasingh, ShipraJaidka, Rani Somani. Comparative clinical evaluation of chemo mechanical caries removal agent Papacarie with conventional method among rural population in India- in vivo study. Braz J Oral Sci 2011; 10(3):193-8.
27. Bohari MR, Chunawalla YK, Ahmed BM. Clinical evaluation of Caries removal in primary teeth using Conventional, Chemo-mechanical and Laser Technique: An in vivo study. J Contemp Dent Pract 2012;13(1):40-7.
28. Hoshino E. Predominant obligate anaerobes in human carious dentin. J Dent Res 1985;64:1195–8.
29. Nancy J, Dorignac G. Lactobacilli from the dentin and saliva in children. J Clin Pediatr Dent 1992;16:107–11.
30. Kidd EA, Ricketts DN, Beighton D. Criteria for caries removal at the enamel-dentine junction: a clinical and microbiological study. Br Dent J. 1996;180:287–91.

Corresponding Author:
Dr. Venkatesh Babu NS.
Department of Pedodontics and Preventive Dentistry,
V.S. Dental College and Hospital,
K.R. Road, V.V. Puram, Bangalore 560004
KARNATAKA, India.
Tel: +9109448710392
Email: drnsvbabu@gmail.com