RESEARCH ARTICLE

Effect of an Oral Health Preventive Protocol on Salivary Parameters and Gingival Health of Children with Type 1 Diabetes

Vishwendra Singh[1], Krishan Gauba[2], Ashima Goyal[3], Devi Dayal[4], Savita Verma[5], GS Prasad[6]

Abstract

Context: Type 1 diabetic children exhibit poorer oral health than general population. However, no oral health preventive protocol exists for attending to the oral health needs of such children.

Aim: To evaluate the effect of an oral health preventive protocol on salivary parameters and gingival health of children with type 1 diabetes mellitus over a period of 6 months.

Materials and methods: Fifty diabetic children, aged 6–12 years were selected and divided into two groups. Children in group I received a comprehensive oral health preventive protocol. The parameters recorded were oral hygiene practices, salivary flow rate, pH, buffer capacity, viscosity, electrolytes, and plaque and gingival indices. These were compared at baseline, 3-, and 6-month intervals.

Statistical analysis: Statistical analysis was done using IBM SPSS STATISTICS (version 22.0). Tests were based on the type of data.

Results: The intervention group (group I) showed favorable improvements in the parameters assessed. A greater number of participants adopted the correct oral hygiene methods. Unstimulated salivary flow rate increased from 0.36 ± 0.21 to 0.82 ± 0.16 mL/minute in group I and from 0.32 ± 0.24 to 0.58 ± 0.16 mL/minute in group II after 6 months ($p = 0.001$). Salivary buffer capacity increased from 3.07 ± 2.64 to 10.40 ± 0.82 in group I while in group II, it improved from 3.20 ± 1.47 to 9.33 ± 1.44 ($p = 0.02$). Salivary viscosity decreased in group I from 1.97 ± 0.42 to 1.15 ± 0.06 and from 1.97 ± 0.35 to 1.23 ± 0.11 in group II after 6 months ($p = 0.02$). Gingival scores changed from 1.07 ± 0.35 to 0.20 ± 0.23 in group I and from 1.04 ± 0.28 to 0.85 ± 0.25 in group II ($p = 0.001$).

Conclusion: The preventive protocol used in the present study showed a significant ($p < 0.05$) improvement in the parameters assessed.

Keywords: Children, Oral health, Preventive, Saliva, Type 1 diabetes mellitus.

International Journal of Clinical Pediatric Dentistry (2021): 10.5005/jp-journals-10005-1871

Introduction

Diabetes mellitus is one of the most common systemic diseases affecting mankind.[1] Type 1 diabetes mellitus (T1DM) accounts for 5–10% of all diagnosed cases of diabetes. The prevalence of juvenile diabetes (onset below 15 years), in India, ranges from 0.8 to 3.61%.[2]

Poorly controlled diabetic children exhibit a higher gingival index, plaque index, and salivary glucose concentration together with a decreased salivary flow rate[3] and salivary pH.[4] As such these children fall in a high dental caries risk category as per the American Academy of Pediatric Dentistry (AAPD, 2008).[5]

Adding to the problem is the way diabetes management has changed over the years. The current concept in diabetic care of children with blood glucose monitoring and frequent injections of short-acting insulin allows a less restricted diet which promotes deleterious oral conditions. Thus management of these children requires a specially designed, systematic, scientific dental preventive protocol, which ensures a rational individualized therapy for them.

Various investigators have demonstrated the effectiveness of preventive instructions and procedures in mitigating dental caries, gingivitis, and plaque scores and have even recommended that routine oral health preventive strategies be used for ameliorating oral health problems in otherwise healthy children.[6–10] However, no such protocol exists for attending to the oral health needs of children with type 1 diabetes.

[1]Department of Oral Health Centre, Government Medical College and Hospital, Chandigarh, India

[2]Oral Health Sciences Centre, Postgraduate Institute of Medical Education and Research, Chandigarh, India

[3]Unit of Pedodontics and Preventive Dentistry, Oral Health Sciences Centre, Chandigarh, India

[4,5]Department of Pediatric Medicine, Advanced Pediatric Centre, Postgraduate Institute of Medical Education and Research, Chandigarh, India

[6]Department of Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India

Corresponding Author: Vishwendra Singh, Department of Oral Health Centre, Government Medical College and Hospital, Chandigarh, India, Phone: +91 8728947189, e-mail: vishwendra89@gmail.com

How to cite this article: Singh V, Gauba K, Goyal A, *et al*. Effect of an Oral Health Preventive Protocol on Salivary Parameters and Gingival Health of Children with Type 1 Diabetes. Int J Clin Pediatr Dent 2021;14(1):109–114.

Source of support: Nil

Conflict of interest: None

Thus, this study was conducted with the following objectives:

- Evaluate the effect of an oral health preventive protocol, over and above the conventional treatment for type 1 diabetes, on salivary parameters—flow rate, pH, buffer capacity, viscosity,

electrolytes, and plaque and gingival indices of children with T1DM over a period of 6 months.
- To assess changes in oral hygiene practices—toothbrushing technique, frequency, and use of fluoridated dentifrice over a period of 6 months.

Materials and Methods

Ethical clearance was obtained from the Institute Ethical Committee of Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh before the initiation of the study (Reference Number: NK/2416/ MDS 12945-46).

The sample size required for this hospital-based study was calculated using Cochran formula. Fifty newly diagnosed (within a week) children with T1DM in the age range of 6–12 years who attended the diabetic clinic at the Advanced Pediatric Centre (APC), PGIMER were selected and divided into two groups (group I and group II) using block randomization. This was done to minimize the effect of treatment for diabetes on their oral health status. The glycated hemoglobin (HbA1c) levels of the participants were obtained from hospital records.

Inclusion Criteria

- Newly diagnosed children with T1DM.
- The age range of 6–12 years.
- Children who gave written assent and whose parents provided written informed consent for participation in the study.

Exclusion Criteria

- Known systemic problems are other than diabetes mellitus.
- History of antibiotic intake in the last 1 month.
- Use of medications like antipsychotics, anticholinergics, anti-secretagogues, etc.

Group I had 25 children who received a comprehensive oral health preventive protocol, followed in the Preventive Dentistry Clinic at PGIMER, the details of which are given in Table 1. The rationale for various steps in the protocol has been discussed later. This preventive program was carried out when the patients showed up for their first dental visits within a span of two months. The 3- and 6-month follow-ups were used to motivate and reinforce the instructions.

Children in group II received the usual diabetes treatment but no preventive protocol was provided to them. However, they were given oral hygiene instructions and the required dental treatment.

Oral Examination Steps

A single examiner (VS) carried out the examination using a mouth mirror, William's probe, and cotton rolls. The collection of saliva was done between 8 and 11 am in Coachman position with passive drooling for a period of 5 minutes. The total quantity of saliva thus collected was divided by 5 to obtain the unstimulated salivary flow rate (USFR) per minute.

Salivary pH and buffer capacity were evaluated using commercially available kits (Saliva Check buffer kits, GC Corp., USA). Participants were asked to expectorate saliva into the saliva collection cup, provided in the kits. The pH and buffer strips were dipped into saliva collected in the cup and removed immediately. Color change was noted within 10 seconds for pH and within 2 minutes for buffer capacity and compared to the charts provided in the kit.

Salivary viscosity was evaluated using Ostwald's viscosimeter while salivary electrolytes—sodium, potassium, chloride, and calcium were measured using SIEMENS auto-analyzer (Dimension RXL Max, Siemens, USA).

Gingival and plaque scores were recorded using the Loe and Silness index[12] and Silness and Löe index,[13] respectively.

A questionnaire was used to gauge the percentage of participants using the oral hygiene practices taught in the study at baseline and subsequent follow-ups.

These parameters were recorded for the patients at baseline, and at 3-, and 6-month intervals. A summary of the methodology has been shown in Flowchart 1.

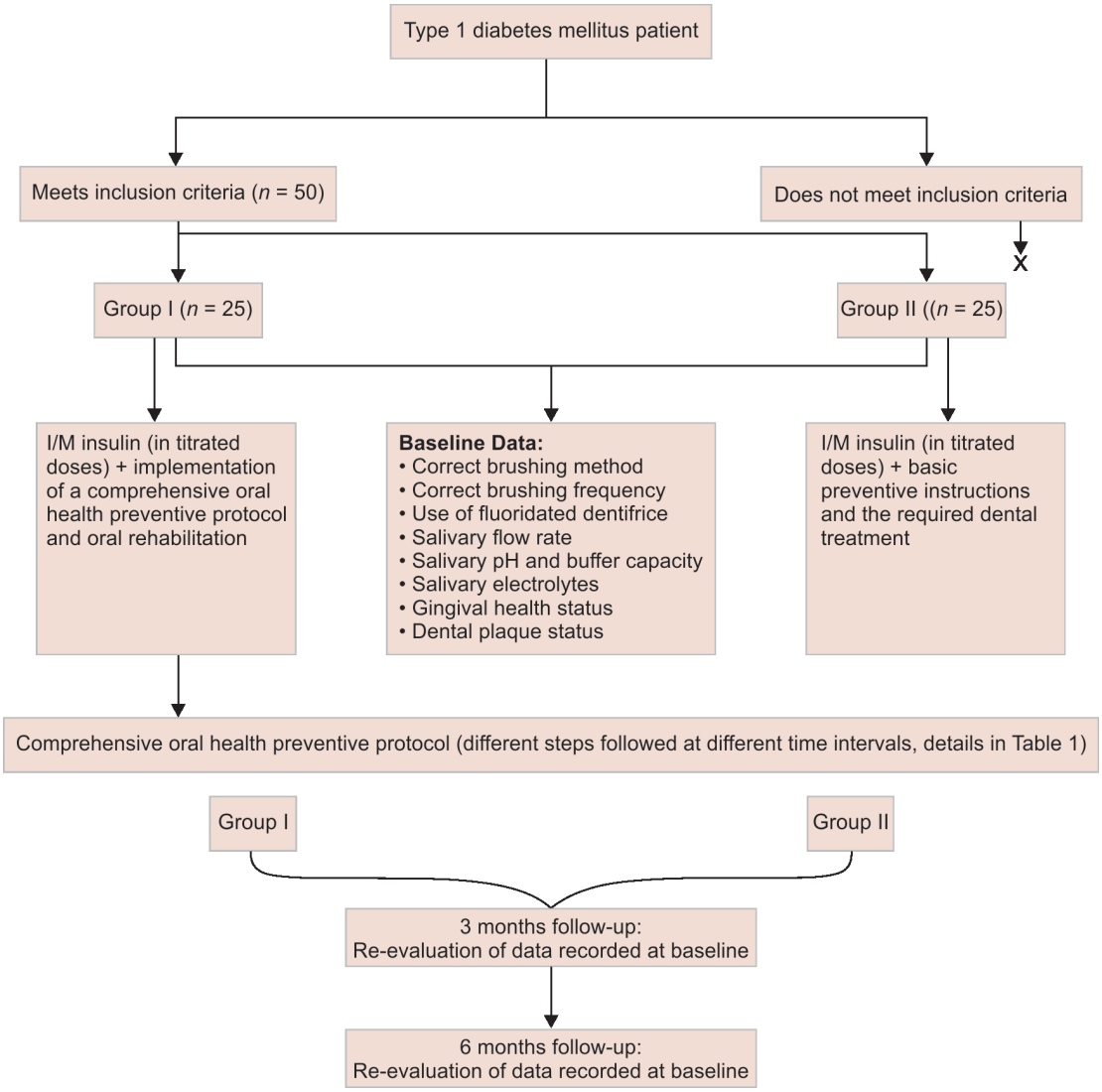
Statistical Analysis

Statistical analysis was done using IBM SPSS STATISTICS (version 22.0). The normality of quantitative data was checked by measures of Kolmogorov–Smirnov tests of normality. For skewed data, comparisons were made by the Mann–Whitney test. For normally distributed data, Student's *t*-test was applied to compare two groups. For time-related variables of skewed data, Wilcoxon signed-rank test was applied. One-way analysis of variance (ANOVA) followed by *post hoc* multiple comparison tests was carried out for normally distributed data. All statistical tests were two-sided and performed at a significance level of $\alpha = 0.05$.

Table 1: Details of the oral health preventive protocol followed in the present study

Appointment 1 (of approximately 40-minute duration)	*Appointment 2 (10 days after the first appointment) (of approximately 45-minute duration)*	*Appointment 3 (15 days after the second appointment) (of about 40-minute duration)*	*Appointment 4 (1 month after the third appointment) (of about 30-minute duration)*
(1) Explanation of the dental problem and concept of prevention (2) Recording of salivary flow rate (Kerr, 1961)[11] (3) Recording of gingival health status (Loe and Silness index, 1963)[12] (4) Recording of plaque scores (Silness and Loe, 1964)[13] followed by brushing demonstration (5) Sealing of open carious lesions (6) Recording of 24-hour diet diary (retrospective collection)	(1) Explanation of the disease process to the child and parent (2) Application of 10% povidone-iodine and 2% topical sodium fluoride varnish on all erupted teeth (3) Discussion of gingival and plaque index scores with the child and the parent	(1) Dietary counseling based upon diet evaluation and analysis of the recorded diet diary (2) Recording of gingival health status (Loe and Silness, 1963) (3) Recording of plaque scores (Silness and Loe, 1964) (4) Oral prophylaxis (5) Continuation of endodontic and restorative procedures already initiated	(1) Recording of salivary flow rate (Kerr, 1961) (2) Recording of the gingival bleeding index (Loe and Silness, 1963) (3) Recording of plaque index (Silness and Loe, 1964)

Flowchart 1: Flowchart depicting the stepwise methodology



Results

The study participants were sex- and age-matched at baseline. Group I had 13 male and 12 female while group II had 14 male and 11 female participants ($p = 0.7$). The mean age of the patients in group I was 8.92 ± 2.040 years and that in group II was 9.68 ± 2.036 years ($p = 0.19$).

Changes observed in hygiene practices and oral parameters of the study participants have been summarized in Table 2. As can be seen, there is an improvement in the oral hygiene practices with more participants adopting the recommended methods. Also, improvement in salivary parameters and plaque and gingival indices is observed over the course of the study. The improvement is, however, greater in participants of group I as compared to group II. Table 3 depicts changes in the levels of salivary electrolytes which have been discussed later.

Discussion

The study evaluated the effect of a comprehensive oral health preventive protocol, over and above the effect of treatment for diabetes, in improving the salivary parameters and gingival health of children with type 1 diabetes. This, to the best of our knowledge, is the first study of this kind.

Different authors have compared type 1 diabetic children with healthy controls and have reported less USFR, salivary pH, salivary buffer capacity, and deranged salivary electrolytes in diabetic children as compared to controls.[14–16] Researchers have also evaluated different types of preventive regimens in healthy children and reported that there was a significant reduction in *mutans* Streptococci counts,[17] improvements in plaque scores,[6,8,9] and dental caries status[6,10] after implementation of the preventive protocol. However, no one has evaluated the efficacy of an oral health preventive protocol in children with type 1 diabetes.

Diabetic children are at an increased risk of poor oral health status. Also, oral health preventive strategies have not yet been evaluated in such children by any investigator. A standardized oral health preventive protocol, which was followed and implemented for participants in group I, is already in use in the Unit of Pedodontics and Preventive Dentistry, PGIMER for children at high risk of dental caries. It has scientifically delineated and sequentially spaced appointments. It consists of four appointments and each appointment involves definite steps as mentioned in

Table 2: Changes in the tested parameters over the course of the study

Parameter assessed	*Group*	*Baseline ($\bar{X} \pm SD$)*	*p value*	*3 months ($\bar{X} \pm SD$)*	*p value*	*6 months ($\bar{X} \pm SD$)*	*p value*
Correct brushing method (modified bass method) (% of participants)	Group I	0	–	66.7	0.01	86.7	0.03
	Group II	0		40		66.7	
Correct brushing frequency (twice daily or after every meal) (% of participants)	Group I	26.7	0.35	100	0.001	100	0.002
	Group II	23.3		40		68	
Use of fluoridated dentifrice (% of participants)	Group I	0	–	93.3	0.002	100	0.001
	Group II	0		40		40	
Unstimulated salivary flow rate (in mL/min)	Group I	0.36 ± 0.21	0.23	0.58 ± 0.15	0.01	0.82 ± 0.16	0.001
	Group II	0.32 ± 0.24		0.46 ± 0.17		0.58 ± 0.16	
Salivary pH	Group I	6.94 ± 0.33	0.95	7.65 ± 0.11	0.01	7.65 ± 0.09	0.10
	Group II	7.00 ± 0.47		7.42 ± 0.26		7.58 ± 0.27	
Salivary buffer capacity	Group I	3.07 ± 2.64	0.86	10.40 ± 1.72	0.005	10.40 ± 0.82	0.02
	Group II	3.20 ± 1.47		8.53 ± 1.59		9.33 ± 1.44	
Salivary viscosity	Group I	1.97 ± 0.42	1.00	1.17 ± 0.06	0.005	1.15 ± 0.06	0.02
	Group II	1.97 ± 0.35		1.29 ± 0.13		1.23 ± 0.11	
Plaque index	Group I	1.41 ± 0.33	0.80	1.04 ± 0.53	0.01	0.36 ± 0.21	0.01
	Group II	1.39 ± 0.25		1.30 ± 0.22		0.90 ± 0.19	
Gingival index	Group I	1.07 ± 0.35	0.40	0.74 ± 0.46	0.01	0.20 ± 0.23	0.001
	Group II	1.04 ± 0.28		1.0 ± 0.24		0.85 ± 0.25	
Mutans Streptococci counts ($\times 10^4$ CFU/mL)	Group I	10.52 ± 12.58	0.40	4.35 ± 2.82	0.03	1.22 ± 0.38	<0.001
	Group II	10.29 ± 22.87		9.5 ± 8.60		7.32 ± 10.51	

Table 3: Comparison of levels of salivary electrolytes at baseline and post-intervention

Electrolyte	*Group*	*Baseline ($\bar{X} \pm SD$)*	*p value*	*3 months ($\bar{X} \pm SD$)*	*p value*	*6 months ($\bar{X} \pm SD$)*	*p value*
Sodium (mmol/L)	Group I	31.72 ± 11.32	1.00	23.68 ± 5.07	0.08	23.96 ± 3.28	0.09
	Group II	32.21 ± 13.44		24.36 ± 4.33		22.08 ± 4.08	
Potassium (mmol/L)	Group I	28.11 ± 8.51	0.98	22.72 ± 3.86	0.22	21.51 ± 2.67	0.47
	Group II	29.02 ± 8.40		25.40 ± 4.11		20.96 ± 2.68	
Chloride (mmol/L)	Group I	35.64 ± 10.59	0.95	23.60 ± 5.54	0.04	21.68 ± 5.61	0.11
	Group II	34.90 ± 8.88		26.44 ± 4.18		24.04 ± 4.81	
Calcium (mg/dL)	Group I	3.40 ± 1.84	0.98	6.77 ± 1.50	0.04	6.09 ± 0.81	0.14
	Group II	3.39 ± 1.80		5.92 ± 1.47		6.80 ± 1.39	

Table 1. The main purpose of the first appointment is to discuss the importance of preventive dentistry and the oral diseases children suffer from with the parents/guardians and the child (patient). The second appointment was scheduled at an interval of 10 days. This duration allows for sufficient time to seal all open carious lesions with intermediate restorative material (IRM) before the second appointment to reduce bacterial load in the oral cavity. It also gave sufficient time to the child to acquaint himself/herself with the proper use of a toothbrush. The second appointment was about explaining to the child/parent/guardian about the specific disease process logically and scientifically. The third appointment involved dietary counseling. However, this was only superficial guidance as the participants were diabetic children and their diets are dictated by their glycemic status. Also, they received a more comprehensive dietary guideline for appropriate management of their blood sugar levels by a trained dietician at the APC, PGIMER. This was done at an interval of 15 days from the second appointment as this duration was sufficient to record any change in the gingival status of the participants. The fourth preventive appointment was kept after a period of 1 month following the third appointment. Within this period, all the rehabilitative work including restorations, endodontic treatment, crowns, etc., was carried out. Also, it is a well-established fact that it takes 4 weeks for a microbial lag phase to occur following diet counseling. This was another reason for keeping a 1-month interval between the third and fourth appointments.

This protocol has already been shown to improve the oral health status in high dental caries risk children.[18] Thus, the implementation of this protocol in the management of diabetic children was expected to improve their oral health too. Though other researchers have proposed 4–10 appointments based oral health preventive protocols,[19–22] these suffer from drawbacks like too closely or irrationally spaced appointments, no provision to test the efficacy of diet counseling, no recording of gingival or plaque indices.

A significantly greater number of children in group I started practising the recommended oral hygiene measures as is evident in Table 2. The brushing method taught in the study was the modified Bass method and was considered as the right method. All other methods like *dattun, manjan*, tooth powder, plain water,

mouthwash, manual toothbrush, powered toothbrush, toothbrush and interdental brush, toothbrush-floss and interdental brush, or any other were considered incorrect from the present study's point of view. Correct brushing frequency was defined as twice daily or brushing after every meal. All other toothbrushing habits like rarely/sometimes, once daily, alternate days, or any other were considered incorrect. This shows that education and motivation about oral hygiene, done at regular intervals, can improve practices adopted by children with diabetes.

The greater increase in the USFR in group I was perhaps partly possible because of the improvement in the glycemic status of the participants. Though, the oral rehabilitative work and preventive regimen had a greater role to play as the children in group I had a greater increase in USFR as compared to the children in group II. Oral rehabilitation leading to a better masticatory efficiency and less inflammation in the oral tissues, a decrease in plaque and hence gingivitis (due to better brushing technique and frequency), a decrease in oral bacteria leading to a reduction in extracellular polysaccharides (due to the use of 10% polyvinylpyrrolidone iodine and fluoride varnish)[23–26] appear to be responsible for the observed results. The relatively less improvement in group II could be because of the lack of regular reinforcement of the preventive instructions carried out in them. Group I also experienced an increase in salivary pH and buffer capacity as compared to group II. It was achieved not just through better metabolic control of diabetes but also due to the preventive protocol as mentioned above.

Salivary viscosity displayed a similar trend as salivary pH with a group I exhibiting a greater reduction in viscosity than group II. A greater salivary flow rate coupled with reduced extracellular polysaccharides and improvement in the basement membrane integrity of salivary gland acini leading to less fluid leakage could have led to an improvement in salivary viscosity.

Salivary electrolytes viz. sodium, potassium, chloride, and calcium do not exhibit the usual concentration pattern in type 1 diabetics due to peripheral vascular and acinic cell membrane damage.[27] An improvement and normalization of these in both the groups, in the present study, was probably due to improved basement membrane integrity as a result of better metabolic control.

Plaque and gingival scores followed similar trends with group I experiencing a greater decrease in scores than group II. These results were an outcome of the preventive package implemented in group I which included education, training, and periodic reinforcement of the correct brushing technique and frequency, use of fluoridated toothpaste which is known to reduce plaque and hence gingivitis,[28] and the use of povidone-iodine which is known to reduce oral microbes[29] and hence dental plaque formation. Also, improvement in the salivary flow rates led to better clearance of debris from the oral cavity which reflected in the reduced amount of dental plaque accumulation. Gingival inflammation subsides if the primary irritant—dental plaque is removed.[30] Hence, gingivitis in the experimental group decreased as a consequence of regular and thorough plaque removal.

The findings of the study are in agreement with Dutta[18] who worked with healthy, non-diabetic children using the same preventive protocol and reported improvement in the salivary flow rate, viscosity, and microbial counts. Researchers have compared type 1 diabetic children with healthy controls in cross-sectional studies and their observations have been mentioned previously. However, a comparison of our work with other researchers was not possible as no one, to the best of our knowledge, has worked with type 1 diabetic children using the oral health preventive protocol as used in the present study.

The study was conducted for a limited period which is a limitation. It is reminded that studies with longer follow-ups and greater sample size be done to establish the benefits of this oral health preventive protocol in children with T1DM.

Conclusion

There was a significant improvement in the salivary parameters and gingival health of children with type 1 diabetes, who usually ignore their oral health on the pretext of their systemic illness, after implementation of an oral preventive protocol. This shows the importance of interdisciplinary cooperation in the management of such children. It is recommended that such a preventive program be included in the medical management of children with T1DM.

References

1. Alamo SM, Soriano YJ, Perez MGS. Dental considerations for the patient with diabetes. Clin Exp Dent 2011;3(1):e25–e30. DOI: 10.4317/jced.3.e25.
2. Amutha A, Thai K, Viswanathan M. Childhood and adolescent onset type 1 diabetes in India. MGM. J Med Sci 2013;1(1):46–53.
3. Twetman S, Nederfors T, Aronson BSS. Two year longitudinal observations of salivary status and dental caries in children with insulin-dependent diabetes mellitus. Pediatr Dent 1992;14(3):184–188.
4. Kennath AAS, Sanjay R, Peramachi P. Evaluation of correlation between salivary Ph and prevalence of dental caries in subjects with and without diabetes mellitus. Res J Rec Sciences 2014;3:224–226.
5. McDonald RE, Avery DR, Dean JA. Dentistry for the child and adolescent. 9th ed., Missouri: Mosby Inc; 2011.
6. Badersten A, Egelberg J, Koch G. Effect of monthly prophylaxis on caries and gingivitis in schoolchildren. Community Dent Oral Epidemiol 1975;3(1):1–4. DOI: 10.1111/j.1600-0528.1975.tb00271.x.
7. Barenie JT, Leske GS, Ripa LW. The effect of toothbrushing frequency on oral hygiene and gingival health in schoolchildren: reassessment after two and one-half years. J Public Health Dent 1976;36(1):9–16. DOI: 10.1111/j.1752-7325.1976.tb02834.x.
8. Bellini HT, Campi R, Denardi JL. Four years of monthly professional tooth cleaning and topical fluoride application in Brazilian school children-effect on gingivitis. J Clin Periodontol 1981;8(3):231–238. DOI: 10.1111/j.1600-051X.1981.tb02034.x.
9. Hamp SE, Lindhe J, Fornell J, et al. Effect of a field program based on systematic plaque control on caries and gingivitis in schoolchildren after 3 years. Community Dent Oral Epidemiol 1978;6(1):17–23. DOI: 10.1111/j.1600-0528.1978.tb01112.x.
10. Rong WS, Bian JY, Wang WJ, et al. Effectiveness of an oral health education and caries prevention program in kindergartens in China. Community Dent Oral Epidemiol 2003;31(6):412–416. DOI: 10.1046/j.1600-0528.2003.00040.x.
11. Stephen KW, Speirs CF. Methods for collecting individual components of mixed saliva: the relevance to clinical pharmacology. Br J Clin Pharmacol 1976;3(2):315–319. DOI: 10.1111/j.1365-2125.1976.tb00609.x.
12. Loe H, Theilade E, Jensen SB. Experimental gingivitis in man. J Periodontol 1965;36(3):177–187. DOI: 10.1902/jop.1965.36.3.177.
13. Silness J, Löe H. Periodontal disease in pregnancy II. correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22(1):121–135. DOI: 10.3109/00016356408993968.
14. Pratibha KM, Johnson P, Ganesh M, et al. Evaluation of salivary profile among adult type 2 diabetes mellitus patients in South India. J Clin Diag Res 2013;7(8):1592–1595. DOI: 10.7860/JCDR/2013/5749.3232.

15. Busato IMS, Igna´cio SA, Brancher JA, et al. Impact of clinical status and salivary conditions on xerostomia and oral health-related quality of life of adolescents with type 1 diabetes mellitus. Community Dent Oral Epidemiol 2012;40(1):62–69. DOI: 10.1111/j.1600-0528.2011.00635.x.
16. Lasisi TJ, Fasanmade AA. Salivary flow and composition in diabetic and non-diabetic subjects. Niger J Physiol Sci 2012;27:79–82.
17. Kristoffersson K, Axelsson P, Bratthall D. Effect of a professional tooth cleaning program on interdentally localized *Streptococcus mutans*. Caries Res 1984;18(5):385–390. DOI: 10.1159/000260792.
18. Dutta S. Development of a preventive module with a scientific rationale for individualized therapy of caries active children [unpublished thesis]. Chandigarh: Panjab University; 1988.
19. Anderson JL. Integration of plaque control into the practice of dentistry. Dent. Clin. North Am 1972;16:621–630.
20. Brandli WE, McCartney MJ, Proctor N. Education in preventive pediatric dentistry. J Dent Child 1979;46:200–203.
21. Koch G, Modeer T, Poulsen S, et al. Text book of pedodontic – a clinical approach. 1st ed., Copenhagen: Munksgard; 1997. pp. 140–141.
22. Evans RW, Clark P, Jia N. The caries management system: are preventive effects sustained post clinical trial? Community Dent Oral Epidemiol 2016;44(2):188–197. DOI: 10.1111/cdoe.12204.
23. Tut OK, Milgrom PM. Topical iodine and fluoride varnish combined is more effective than fluoride varnish alone for protecting erupting first permanent molars: a retrospective cohort study. J Public Health Dent 2010;70(3):249–252. DOI: 10.1111/j.1752-7325.2010.00163.x.
24. Featherstone JD. Delivery challenges for fluoride, chlorhexidine and xylitol. BMC Oral Health 2006;6(Suppl1):S8. DOI: 10.1186/1472-6831-6-S1-S8.
25. Milgrom P, Weinstein P, Huebner C, et al. Empowering head start to improve access to good oral health for children from low income families. Matern Child Health J 2008;15(7):876–882. DOI: 10.1007/s10995-008-0316-6.
26. Twetman S, Fritzon B, Jensen B, et al. Pre- and post-treatment levels of salivary mutans Streptococci and lactobacilli in pre-school children. Inter J Paediatr Dent 1999;9(2):93–98. DOI: 10.1046/j.1365-263x.1999.00108.x.
27. Marder MZ, Abelson DC, Mandel ID. Saliva alteration in diabetes mellitus. J Periodontol 1975;46(9):567–570. DOI: 10.1902/jop.1975.46.9.567.
28. Bay I, Rolla G. Plaque inhibition and improved gingival condition by use of a stannous fluoride toothpaste. Scand J Dent Res 1980;88(4):313–315. DOI: 10.1111/j.1600-0722.1980.tb01232.x.
29. Amin SM, Harrison RL, Benton TS, et al. Effect of povidone-iodine on *Streptococcus mutans* in children with extensive dental caries. Pediatr Dent 2004;26:5–10.
30. Listgarten MA. Plaque and periodontal disease. J Clin Periodontol 1988;15(8):485–487. DOI: 10.1111/j.1600-051X.1988.tb01019.x.