Clinical Study

Enumeration of Salivary Streptococci and Lactobacilli in Children with Differing Caries Experiences in a Rural Indian Population

Sreeja Ravindran,[1] Minal Chaudhary,[2] and Madhuri Gawande[2]

[1] *Ibn Sina National College for Medical Studies, Al Mahjar, Jeddah 21418, Saudi Arabia*
[2] *Department of Oral Pathology and Microbiology, Sharad Pawar Dental College and Hospital, Sawangi (Meghe), Wardha, Maharashtra 442001, India*

Correspondence should be addressed to Sreeja Ravindran; sree.dent@rediffmail.com

Objectives. Compare the total salivary Streptococci and Lactobacilli counts in cleft and noncleft children with differing caries experiences, correlate the bacterial counts with dmft/DMFT status and identify the different biotypes of Mutans Streptococci (MS). *Patients*. Group I included thirty subjects with dental caries (DC) and cleft lip and palate (CL/P); Group II had thirty subjects with DC but without CL/P. Group III comprised a control of thirty subjects with neither DC nor CL/P. *Methodology*. Enumeration of total salivary Streptococci and Lactobacilli was done by the plate count method and correlation of counts with dmft/ DMFT status examined. Differences in biochemical reactions were used to identify the biotypes. *Results*. Streptococci colonies in CL/P children with caries (64.30 ± 24.52) was significantly higher than in children with no CL/P or caries (45.57 ± 16.73). No significant differences in the Lactobacilli count were observed. dmft/DMFT status and Streptococci counts showed a strong positive correlation whereas Lactobacilli counts showed a moderate correlation. *S. mutans* was the predominant biotype. *Conclusions*. Higher total salivary Streptococci and Lactobacilli counts exist in cleft subjects with caries than in the non-cleft subjects. Positive correlation between dmft/DMFT scores and salivary Streptococci reinforces its role in DC. *S. mutans* and *S. sobrinus* are the biotypes more frequently associated with dental caries in children.

1. Introduction

Dental caries is a complex process shrouded by many indirect factors which obscure the direct causes. Bacteria are thought to play an integral role in this process. Cariogenic microorganisms colonize on tooth surfaces, cause a marked reduction of pH in the presence of a sugar substrate, and consequently induce dental caries. Studies have shown that Streptococcus mutans is an efficient cariogenic microorganism [1, 2]. Most populations with a high caries experience have also reported positive associations between caries experience and salivary levels of Lactobacilli [3, 4].

CL/P individuals, in addition to the presence of anatomic and functional deficiencies, also have a higher prevalence of dental disease than normal children. The literature on CL/P subjects and dental caries is conflicting with reports suggesting that individuals born with oral clefts have a higher

risk of caries [5–11] and there also exist studies that did not find any difference in caries experience between individuals born with clefts and unaffected controls [12–16]. A recent meta-analysis on the frequency of caries in individuals born with clefts was inconclusive [17].

Among the cariogenic microbes, the roles of Streptococci and Lactobacilli have been investigated in CL/P subjects as well [18, 19]. In addition to a sugar rich diet, poor oral hygiene maintenance, lack of motivation to perform regular preventive dental home care, low fluoride exposure, and malaligned teeth have been put forward as possible explanations [20]. Since it is not possible to encompass all these factors in a limited time frame study, we focused on the vital microbial aspect of the caries process. Most studies on the microbial flora of CL/P patients have been carried out in developed countries, with little microbial data available from an Indian population [21–23]. Therefore, we compared

TABLE 1: Scheme of biochemical tests (adapted from references [25, 37]).

Species	Hydrolysis of arginine	Fermentation of sugars					Aerobic growth	Bacitracin resistance
		Mannitol	Sorbitol	Inulin	Mellibiose	Raffinose		
S. mutans	−	+	+	+	+	+	+	+
S. rattus	+	+	+	+	+	+	+	+
S. cricetus	−	+	+	+	+	+	−	−
S. sobrinus	−	+	±	+	−	−	+	+

the salivary Streptococci and Lactobacilli counts of cleft and noncleft children with differing caries experience in a rural Indian population and correlated with the dmft/DMFT status.

Mutans Streptococci (MS) have been grouped into six biotypes of which four, namely, *S. mutans*, *S. sobrinus*, *S. cricetus*, and *S. rattus* have been identified as the primary hosts in humans [24, 25]. Some bacterial strains are more likely to be associated with the caries process than others. Hence, we also investigated the different MS biotypes present in the cleft and noncleft children in order to gain a better insight into the caries process.

2. Materials and Methods

The study was set in Department of Oral Maxillofacial Pathology and Microbiology in a rural part of central India. Examination for dental caries in all the subjects was carried out by a single examiner under natural light using a mouth mirror and explorer. Sum of decayed, missing due to caries and filled teeth in the deciduous dentition (dmft) and sum of decayed, missing due to caries and filled teeth in the permanent dentition (DMFT) were used to assess caries experience as per the WHO criteria [26]. Caries prevalence (dmft/DMFT > 0) and severity (mean dmft/DMFT) were calculated.

A total of 90 subjects aged 6–12 years were divided into 3 groups: Group I included 30 subjects with DC and CL/P (4 BCLP, 24 UCLP and 2 CP subjects were enrolled. Presence of labial and/or palatal fistulae was noted in 18 of the 30 subjects), Group II included 30 subjects with DC without CL/P, and Group III included 30 subjects with no DC or CL/P. Group I children were consecutively drawn from subjects reporting for secondary surgical repairs, speech therapy or seeking definitive orthodontic treatment as a part of their CL/P follow-up visits. Non cleft subjects of Groups II and III were consecutively selected from a regular pool of dental patients. Subjects with history of drug therapy three months prior to the saliva sampling, any systemic disease, or undergoing active orthodontic treatment were excluded from the study. Informed consent was obtained from the study subjects or their parents prior to inclusion in the study and clearance obtained from the institutional ethical committee.

2.1. Collection of Saliva. Paraffin-stimulated whole saliva of subjects in the different groups was collected following the protocol previously described [27]. Participants were requested to void saliva into a wide-mouthed disposable cup two hours after their last meal in a mid morning session.

2.2. Culture Method. Saliva (0.5 mL) was diluted with 9.5 mL of sterile water and tenfold serial dilutions of saliva were made up to 10^{-6}, before plating. Mitis Salivarius Agar (MSA) supplemented with potassium tellurite and Rogosa SL were used as culture media for isolation of total salivary Streptococci and Lactobacilli, respectively. Appropriate amounts of semisolid media was poured onto petri-plates in a laminar air flow unit and allowed to solidify at room temperature. To each plate 100 μL of the diluted sample was spread evenly and the plates were sealed, placed in an anaerobic jar with AnaeroGas Pack system (Himedia), and incubated at 37°C for 48 hours. Bacterial colonies were observed under a dissecting microscope and deduced on the basis of colony morphology, shape and color; and in case of doubt confirmation for Streptococci and Lactobacilli was done by gram staining or catalase tests, respectively. Colony counting was done under a colony counter and the number of CFU were multiplied by the number of times the original mL of sample was diluted (the dilution factor of the plate counted) and expressed as the number of colony forming units per milliliter (CFU/mL) of saliva.

2.3. Morphological and Biochemical Identification of Isolates. The colonies showing resemblance to the Mutans Streptococci on the basis of morphologic appearance, size, and color characteristics were subjected to a biochemical scheme of tests (Table 1) for identification and differentiation of the biotypes. Each of the different colony morphologies obtained were four-way streaked on MSA media and incubated in anaerobic gas jar with AnaeroGas pack for 24 hours. The colonies obtained were subcultured on a 7.5 mL of slant containing MSA media for pure isolation and again incubated for 48 hours anaerobically. The pure isolates obtained on the slants were then subjected to the biochemical tests. Strains were distinguished based on their ability to ferment different carbohydrates (Mannitol, Sorbitol, Inulin, Mellibiose, Raffinose), the ability to hydrolyze arginine, aerobic growth, and susceptibility to bacitracin. The biochemical tests were repeated with cultures in order to confirm reproducibility and reliability.

All the data were recorded and subjected to statistical analysis in SPSS 17. Descriptive statistical tests including the mean Streptococci and Lactobacilli counts in the different groups were determined. One-way ANOVA compared the mean salivary Streptococcal counts and Lactobacilli counts in the different groups. Tukey post hoc test was applied to determine the groups in which the significant differences were. Pearson product-moment correlation was run to determine the relationship between dmft/DMFT status of the children

TABLE 2: Caries and age data of the subjects.

Group	dmft/DMFT		Mean dmft/DMFT	Mean age
	1–3	>3		
I	21	9	2.17	10.2
II	22	8	2.17	9.8
III	0	0	0	9.7

and their salivary Streptococci and Lactobacilli numbers. The significance level was established at $P < 0.05$.

3. Results

dmft/DMFT scores of greater than 3 were noted in nine subjects with cleft lip and palate. The twenty-one remaining cleft subjects had dmft/DMFT scores between 1–3. Eight of the noncleft subjects had dmft/DMFT scores more than 3. The remaining twenty-two noncleft subjects had dmft/DMFT scores between 1 and 3. Mean dmft/DMFT score (2.17) in the cleft and noncleft groups was similar (Table 2).

At sample dilutions beyond the concentration factor 10^3 the number of CFU that could be counted tended to be less than 30, hence dilution factor 10^3 was chosen for calculation of the mean Streptococci and Lactobacilli to avoid false positive results. In Table 3, the mean and standard deviations of the salivary Streptococci and Lactobacilli levels in the different groups are detailed; in the last two columns, the results of the statistical analysis are reported. There was a statistically significant difference between the Streptococci count in the different groups as determined by one-way ANOVA. A Tukey post hoc test revealed that the Streptococci count in the group of cleft children with caries (64.3 ± 24.5 CFUs) was significantly higher than in the control subjects without cleft or caries (45.5 ± 16.7 CFUs) ($P = 0.004$). No statistically significant differences were noted in the Streptococci counts in subjects with no clefts but having caries and the control group ($P = 0.459$). Mean Lactobacilli counts of the cleft children with caries (23.8 ± 18.0 CFUs), the subjects with no clefts but having caries (27.1 ± 19.8 CFUs), and control subjects without cleft or caries (18.4 ± 15.8 CFUs) showed no statistical significant difference.

Pearson correlation was used to determine the relationship between dmft/DMFT status of the children and their salivary Streptococci and Lactobacilli numbers. Streptococci count and the dmft/DMFT scores in the subjects with cleft and caries showed a strong, positive correlation ($r = 0.81$). Similarly a strong positive correlation was observed for the Streptococci count and the dmft/DMFT scores for subjects without clefts but having caries ($r = 0.84$). Lactobacilli counts and dmft/DMFT scores in Groups I and II showed a moderate positive correlation ($r = 0.42$ and $r = 0.51$, resp.) (Figures 1 and 2).

In children with both caries and clefts *S. mutans* was identified in nearly two-thirds (63%) of the subjects followed by *S. sobrinus* from 20% of the subjects. In children with no clefts but having decayed teeth *S. mutans* and *S. sobrinus* were the biotypes identified from 53% and 10% of the subjects, respectively. In less than half (40%) of the children from
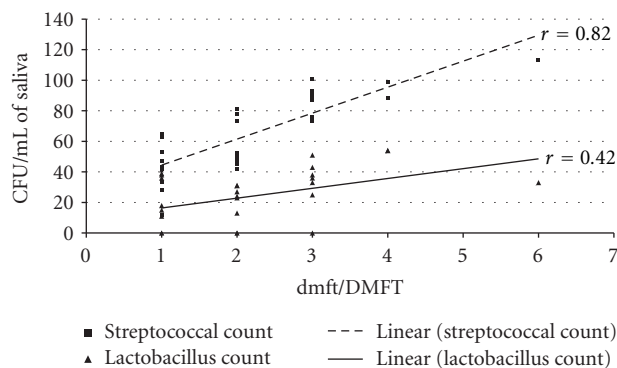

FIGURE 1: Correlation of dmft/DMFT with Streptococci and Lactobacilli in Group I.
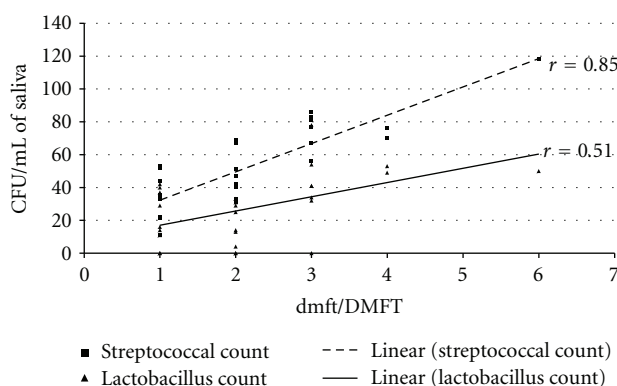

FIGURE 2: Correlation of dmft/DMFT with Streptococci and Lactobacilli counts in Group II.

the control group biotype *S. mutans* was recovered, and in only a small fraction (3.33%) was *S. sobrinus* identified. Interestingly, in none of the subjects *S. sobrinus* was identified in isolation and it was always identified along with *S. mutans*. The biotypes *S. cricetus* and *S. rattus* were not identified in any of the subjects. In certain subjects (60%, 76.6%, and 90% in Groups I, II, and III resp.) biochemical categorization of the strains, although resembling the Mutans group on the basis of morphologic characteristics, was inconclusive (Figure 3).

4. Discussion

Dental caries is a simple process in concept, but complicated in detail and microbes are a vital cog in the wheel of this process. Detection and quantitation of microbes is necessary to understand the disease process. Several molecular methods have been developed for the detection and quantitation of microorganisms on the basis of differences in DNA and RNA; that are simple, rapid in detection, and technically sensitive. Conventional culture has been employed since long for detection and quantitation of microbes and if the strict adherence to procedures is followed such as sterile lab conditions, strict anaerobic/aerobic protocols maintained then conventional plating and enumeration also produces predictive results. However, there are certain drawbacks to the conventional

TABLE 3: Mean salivary Streptococci and Lactobacilli counts in the different groups.

Bacterial count (CFU/mL saliva[*])	Group I mean ± SD	Group II mean ± SD	Group III mean ± SD	ANOVA	Tukey
Streptococci	64.30 ± 24.52	52.37 ± 23.94	45.57 ± 16.73	0.005	0.004 (I & III)
Lactobacilli	23.87 ± 18.01	27.13 ± 19.84	18.43 ± 15.81	NS	

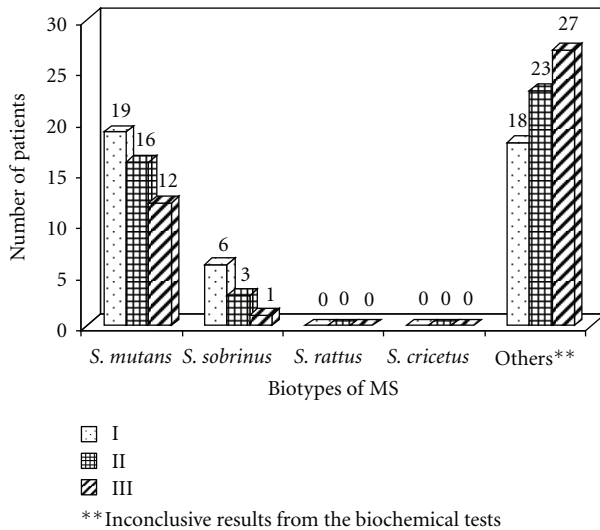[*] Based on mean CFU per sample. To be multiplied by 10^3.

FIGURE 3: Biotypes in the different groups.

methods including long time span, labor intensiveness, and reduced level of sensitivity when compared to the molecular methods [28].

Saliva samples were used in the study to assess the microbial aspect of dental caries. Questions concerning reliability of saliva over plaque or oral swab samples can be raised. The reports of Mundroff et al. (1990) and Dasanayake et al. (1995) nullify this doubt as they found that detection of Streptococci and Lactobacilli in stimulated saliva was in excellent agreement with either pooled plaque or oral swab samples [29, 30]. Studies have shown that differences in culture may result when different media are used [31, 32]. Mitis-Salivarius (MS) agar was developed as a selective and differential medium for oral Streptococci [33]. The addition of Bacitracin and sucrose to MS agar (MSB agar) led to better selective recovery and isolation of S. mutans [34]. However, Schaeken et al. (1986) have shown that addition of bacitracin is inhibitory to certain MS biotypes, especially S. sobrinus and S. cricetus. Chair-side methods exist that are rapid and simple in detection [35, 36]. However they do not permit biotyping of MS. We chose a biochemical scheme based on the papers of Hamada and Slade (1980) and Coykendall (1989) to permit the identification and differentiation of biotypes [25, 37].

Caries experience was assessed using the popular dmft/DMFT index. Both measures were used as the age group of subjects in the study was 6–10 years, and in this age group children having both primary and permanent teeth are present. Interestingly, the caries severity (mean dmft/DMFT) was identical in the cleft and noncleft groups.

It needs to be kept in mind that the use of dmft/DMFT scores is not without limitations. The "M/m" and "F/f" component of the score may be affected not only due to new caries lesions, but also due to dental units missing due to reasons like trauma. Appropriate steps were taken in the study and reasons why teeth may have been extracted were investigated to avoid counting teeth missing or filled for reasons other than caries. Strong positive numerical association was noted between caries experience and Streptococci levels in children having caries, both cleft and noncleft. Studies by Hegde et al. (2005) and Ali et al. (1998) have reported similar results of caries experience and S. mutans levels in Indian populations [23, 38]. In contrast studies by Dasanayake and Caufield (2002) in SriLankan children and Gudkina and Brinkmane. (2008) in Riga failed to find positive correlation between caries and salivary S. mutans levels [39, 40].

The Streptococcal count in CL/P subjects with DC was higher than the counts in non CL/P subjects with DC. Studies have proposed that the presence of oral clefts leads to poor oral hygiene [5–11]. Presence of fistulae was noted in more than half of the CL/P subjects in the study. Cleft subjects with unrepaired fistula are noted to have a poorer oral hygiene status and increased prevalence of caries than fully repaired subjects [7, 41]. Ahluwalia et al. (2004) proposed that the longer clearance time of food from the oral cavity of subjects with palatal clefts was responsible for the high caries prevalence. Though the caries experiences in subjects with and without clefts in our study were similar, the Streptococci counts were higher in the cleft children. Food substrates for the cariogenic bacteria tend to remain for a longer duration in cleft subjects with residual fistulae, due to reasons like nasal regurgitation and food impaction in the fistula. This in turn may probably lead to an increase in colonisation of cariogenic bacteria. Additionally, Turner et al. (1998) suggested that the presence of nasal fluid draining into the oral cavity leads to increased adherence of plaque bacteria onto tooth surfaces. These factors could possibly explain the higher Streptococcal counts in CL/P subjects in the current study.

Lactobacilli are notable organisms in dental caries and clinical studies have shown positive correlation of Lactobacilli number in the plaque and saliva of caries active individuals [3, 4]. Available data suggest that in plaque from the smooth surface of the teeth and as well as in plaque beneath which caries is initiated, Lactobacilli constitute generally low or negligible proportion of plaque microbiota. In contrast, Lactobacilli are found in relatively high proportions in cavitated lesions suggesting that their role in dental caries probably lies in progression and in advanced carious lesion rather than in initiation of the disease [42]. Though a differentiation of carious lesions was not made in the study on the basis of duration, it is possible that a majority of caries active

subjects in the cleft and non cleft groups may have presented with carious lesions that were in advanced stages. This is because many subjects included in this study were from a rural population and the likelihood that they were exposed to timely treatment strategies is minimal. Additionally, visits to the dentist to address concerns related to dental pain were more often resolved through extractions. Lack of timely preventive dental strategies and access to basic dental care for the rural population in this study may possibly be responsible factors.

The human mouth is usually sterile at birth but soon acquires a predominantly streptococcal microbiota and with age the microbiota of streptococcal species differentiates. Rogers (1969), isolated 82 streptococcal strains from the mouth of individuals with active caries [43]. Mutans streptococci are harbored by virtually all dentate humans, albeit in relatively low proportions compared with other oral streptococci. The biotypes identified were *S. mutans* and *S. sobrinus* of which *S. mutans* were recovered in greater numbers. Additionally, *S. sobrinus* was not identified in isolation and was always identified together with *S. mutans* similar to reports of Loesche [44]. Though we failed to recover *S. cricetus* and *S. rattus*, reports of finding these strains have also been published [45].

Some of the colonies when subjected to the biochemical tests failed to show categorical results. Hamada and Slade [37] reported the characteristic colony morphologies *S. mutans* and *S. sobrinus* form when grown on MSA. Presumptive identification of the colonies on the basis of color size and shape characteristics was done initially prior to biochemical testing. However, lack of conclusive results from these colonies in the biochemical scheme is probably because they did not fit into the MS group, although they were initially labeled as MS on the basis of appearance. In an interesting report [46], So et al. recovered non-MS from one-third of the plaque samples they plated on MSB agar, though it is popularly used as a selective medium. No previous literature exists regarding the predominant biotypes of MS in CL/P subjects and so comparison with other studies is not possible.

Caution should be exercised in extrapolation of the results as differences in microbial growth and enumeration tend to result when different microbial methods are chosen. Also differences in bacterial isolation could result due to differences in dilution of salivary samples, tooth brushing prior to visiting the clinic or repeated swallowing leading to clearing of bacteria from saliva [47, 48].

5. Conclusions

Higher streptococcal and lactobacilli counts were observed in subjects with CL/P and DC than in subjects with DC but no CL/P and the least counts were in the caries free subjects. Streptococcal counts showed strong positive correlation with dmft/DMFT whereas Lactobacillus counts were only weakly correlated with dmft/DMFT scores in the groups with dental caries. Positive correlation between dmft/DMFT scores and streptococci probably reinforces its strong association with DC development. Though there was lack of a clear relationship between levels of particular biotypes and DC,

the biotypes of *S. mutans* and *S. sobrinus* were found to be overwhelmingly associated with the subjects with DC.

References

[1] R. J. Fitzgerald and P. H. Keyes, "Demonstration of the etiologic role of streptococci in experimental caries in the hamster," *The Journal of the American Dental Association*, vol. 61, pp. 9–19, 1960.

[2] J. van Houte, "Microbiological predictors of caries risk," *Advances in Dental Research*, vol. 7, no. 2, pp. 87–96, 1993.

[3] I. Zickert, C. G. Emilson, and B. Krasse, "Streptococcus mutans, lactobacilli and dental health in 13-14-year-old Swedish children," *Community Dentistry and Oral Epidemiology*, vol. 10, no. 2, pp. 77–81, 1982.

[4] M. I. Matee, F. H. Mikx, S. Y. Maselle, and W. H. Van Palenstein Helderman, "Mutans streptococci and lactobacilli in breast-fed children with rampant caries," *Caries Research*, vol. 26, no. 3, pp. 183–187, 1992.

[5] D. C. Johnsen and M. Dixon, "Dental caries of primary incisors in children with cleft lip and palate," *Cleft Palate-Craniofacial Journal*, vol. 21, no. 2, pp. 104–109, 1984.

[6] B. Bokhout, F. X. W. M. Hofman, J. van Limbeek, G. J. C. Kramer, and B. Prahl-Andersen, "Increased caries prevalence in 2.5-year-old children with cleft lip and/or palate," *European Journal of Oral Sciences*, vol. 104, no. 5-6, pp. 518–522, 1996.

[7] M. Ahluwalia, S. R. Brailsford, E. Tarelli et al., "Dental caries, oral hygiene, and oral clearance in children with craniofacial disorders," *Journal of Dental Research*, vol. 83, no. 2, pp. 175–179, 2004.

[8] S. Besseling and L. Dubois, "The prevalence of caries in children with a cleft lip and/or palate in Southern Vietnam," *Cleft Palate-Craniofacial Journal*, vol. 41, no. 6, pp. 629–632, 2004.

[9] A. Kirchberg, A. Treide, and A. Hemprich, "Investigation of caries prevalence in children with cleft lip, alveolus, and palate," *Journal of Cranio-Maxillofacial Surgery*, vol. 32, no. 4, pp. 216–219, 2004.

[10] M. Stec-Slonicz, J. Szczepańska, and U. Hirschfelder, "Comparison of caries prevalence in two populations of cleft patients," *Cleft Palate-Craniofacial Journal*, vol. 44, no. 5, pp. 532–537, 2007.

[11] W. C. Zhu, J. Xiao, Y. Liu, J. Wu, and J. Li, "Caries experience in individuals with cleft lip and/or palate in China," *Cleft Palate-Craniofacial Journal*, vol. 47, no. 1, pp. 43–47, 2010.

[12] M. Lausterstein and M. Mendelsohn, "An analysis of the caries experience of 285 cleft palate children," *The Cleft Palate Journal*, vol. 29, pp. 314–319, 1964.

[13] B. Zschieschack and R. Grabowski, "The influence of caries of the deciduous teeth upon development of the dentition in patients with cleft lip, jaw and palate," *Journal of Orofacial Orthopedics*, vol. 60, no. 3, pp. 215–224, 1999.

[14] V. S. Lucas, R. Gupta, O. Ololade, M. Gelbier, and G. J. Roberts, "Dental health indices and caries associated microflora in children with unilateral cleft lip and palate," *Cleft Palate-Craniofacial Journal*, vol. 37, no. 5, pp. 447–452, 2000.

[15] J. Chapple and J. Nunn, "The oral health of children with clefts of the lip, palate, or both," *The Cleft Palate-Craniofacial Journal*, vol. 38, pp. 525–528, 2001.

[16] E. M. Bastos Lages, B. Marcos, and I. A. Pordeus, "Oral health of individuals with cleft lip cleft palate, or both," *Cleft Palate-Craniofacial Journal*, vol. 41, no. 1, pp. 59–63, 2004.

[17] P. Hasslöf and S. Wetman, "Caries prevalence in children with cleft lip and palate—a systematic review of case-control studies," *International Journal of Paediatric Dentistry*, vol. 17, no. 5, pp. 313–319, 2007.

[18] B. Bokhout, C. Van Loveren, F. X. W. M. Hofman, J. F. Buijs, J. Van Limbeek, and B. Prahl-Andersen, "Prevalence of Streptococcus mutans and lactobacilli in 18-month-old children with cleft lip and/or palate," *Cleft Palate-Craniofacial Journal*, vol. 33, no. 5, pp. 424–428, 1996.

[19] C. Van Loveren, J. F. Buijs, B. Bokhout, B. Prahl-Andersen, and J. M. Ten Cate, "Incidence of mutans streptococci and lactobacilli in oral cleft children wearing acrylic plates from shortly after birth," *Oral Microbiology and Immunology*, vol. 13, no. 5, pp. 286–291, 1998.

[20] F. W. L. Wong and N. M. King, "The oral health of children with clefts—a review," *Cleft Palate-Craniofacial Journal*, vol. 35, pp. 248–254, 1998.

[21] A. V. Ankola, L. Nagesh, P. Hegde, and G. N. Karibasappa, "Primary dentition status and treatment needs of children with cleft lip and/or palate," *Journal of Indian Society of Pedodontics and Preventive Dentistry*, vol. 23, no. 2, pp. 80–82, 2005.

[22] R. Krishnakumar, S. Singh, and V. V. S. Reddy, "Comparison of levels of mutans streptococci and lactobacilli in children with nursing bottle caries, rampant caries, healthy children with 3-5 dmft/DMFT and healthy caries free children," *Journal of the Indian Society of Pedodontics and Preventive Dentistry*, vol. 20, no. 1, pp. 1–5, 2002.

[23] P. P. Hegde, B. R. Ashok Kumar, and V. A. Ankola, "Dental caries experience and salivary levels of Streptococcus mutans and Lactobacilli in 13-15 years old children of Belgaum city, Karnataka," *Journal of Indian Society of Pedodontics and Preventive Dentistry*, vol. 23, no. 1, pp. 23–26, 2005.

[24] R. A. Whiley and D. Beighton, "Current classification of the oral streptococci," *Oral Microbiology and Immunology*, vol. 13, no. 4, pp. 195–216, 1998.

[25] A. L. Coykendall, "Classification and identification of the viridans streptococci," *Clinical Microbiology Reviews*, vol. 2, no. 3, pp. 315–328, 1989.

[26] World Health Organization, *Oral Health Surveys Basic Methods*, World Health Organization, Geneva, Switzerland, 4th edition, 1997.

[27] M. Navazesh and S. K. S. Kumar, "Measuring salivary flow," *The Journal of the American Dental Association*, vol. 139, pp. 35–40, 2008.

[28] K. Boutaga, A. J. van Winkelhoff, C. M. J. E. Vandenbroucke-Grauls, and P. H. M. Savelkoul, "Comparison of Real-Time PCR and culture for detection of Porphyromonas Gingivalis in subgingival plaque samples," *Journal of Clinical Microbiology*, vol. 41, no. 11, pp. 4950–4954, 2003.

[29] S. A. Mundorff, A. D. Eisenberg, D. H. Leverett, M. A. Espeland, and H. M. Proskin, "Correlations between numbers of microflora in plaque and saliva," *Caries Research*, vol. 24, no. 5, pp. 312–317, 1990.

[30] A. P. Dasanayake, P. W. Caufield, G. R. Cutter, J. M. Roseman, and B. Kohler, "Differences in the detection and enumeration of mutans streptococci due to differences in methods," *Archives of Oral Biology*, vol. 40, no. 4, pp. 345–351, 1995.

[31] W. A. Little, D. C. Korts, L. A. Thomson, and W. H. Bowen, "Comparative recovery of Streptococcus mutans on ten isolation media," *Journal of Clinical Microbiology*, vol. 5, no. 6, pp. 578–583, 1977.

[32] M. J. Schaeken, J. S. van der Hoeven, and H. C. Franken, "Comparative recovery of Streptococcus mutans on five isolation media, including a new simple selective medium," *Journal of Dental Research*, vol. 65, no. 6, pp. 906–908, 1986.

[33] H. V. Jordan, B. Krasse, and A. Möller, "A method of sampling human dental plaque for certain "caries-inducing" streptococci," *Archives of Oral Biology*, vol. 13, no. 8, pp. 919–927, 1968.

[34] O. G. Gold, H. V. Jordan, and J. van Houte, "A selective medium for Streptococcus mutans," *Archives of Oral Biology*, vol. 18, no. 11, pp. 1357–1364, 1973.

[35] B. Jensen and D. Bratthall, "A new method for the estimation of mutans streptococci in human saliva," *Journal of Dental Research*, vol. 68, no. 3, pp. 468–471, 1989.

[36] D. Bratthall, A. Hoszek, and X. Zhao, "Evaluation of a simplified method for site-specific determination of mutans streptococci levels," *Swedish Dental Journal*, vol. 20, no. 6, pp. 215–220, 1996.

[37] S. Hamada and H. D. Slade, "Biology, immunology, and cariogenicity of Streptococcus mutans," *Microbiological Reviews*, vol. 44, no. 2, pp. 331–384, 1980.

[38] Y. A. Ali, N. J. Chandranee, B. J. Wadher, A. Khan, and Z. H. Khan, "Relationship between caries status, colony forming units (cfu) of Streptococcus mutans and Snyder caries activity test," *Journal of the Indian Society of Pedodontics and Preventive Dentistry*, vol. 16, no. 2, pp. 56–60, 1998.

[39] A. P. Dasanayake and P. W. Caufield, "Prevalence of dental caries in Sri Lankan aboriginal Veddha children," *International Dental Journal*, vol. 52, no. 6, pp. 438–444, 2002.

[40] J. Gudkina and A. Brinkmane, "Caries experience in relation to oral hygiene, salivary cariogenic microflora, buffer capacity and secretion rate in 6-year olds and 12 year olds in Riga," *Stomatologija*, vol. 10, no. 2, pp. 76–80, 2008.

[41] C. Turner, A. F. Zagirova, L. E. Frolova, F. J. Courts, and W. N. Williams, "Oral health status of Russian children with unilateral cleft lip and palate," *Cleft Palate-Craniofacial Journal*, vol. 35, no. 6, pp. 489–494, 1998.

[42] W. P. Holbrook, "Dental caries and cariogenic factors in preschool urban Icelandic children," *Caries Research*, vol. 27, no. 5, pp. 431–437, 1993.

[43] A. H. Rogers, "The proportional distribution and characteristics of streptococci in human dental plaque," *Caries Research*, vol. 3, no. 3, pp. 238–248, 1969.

[44] W. J. Loesche, "Role of Streptococcus mutans in human dental decay," *Microbiological Reviews*, vol. 50, no. 4, pp. 353–380, 1986.

[45] H. C. van der Mei, J. J. de Soet, J. de Graaff, P. G. Rouxhet, and H. J. Busscher, "Comparison of the physicochemical surface properties of Streptococcus rattus with those of other mutans streptococcal species," *Caries Research*, vol. 25, no. 6, pp. 415–423, 1991.

[46] Y. Y. So, S. K. Pyung, H. K. Hwang et al., "Identification of non-mutans streptococci organisms in dental plaques recovering on mitis-salivarius bacitracin agar medium," *Journal of Microbiology*, vol. 43, no. 2, pp. 204–208, 2005.

[47] J. Okpalugo, K. Ibrahim, and U. S. Inyang, "Toothpaste formulation efficacy in reducing oral flora," *Tropical Journal of Pharmaceutical Research*, vol. 8, no. 1, pp. 71–77, 2009.

[48] W. H. Bowen, "Salivary influences on the oral microflora," in *Saliva and Oral Health*, W. M. Edgar and D. M. O'Mullane, Eds., pp. 95–103, London, UK, 1996.

The Scientific
World Journal

Gastroenterology
Research and Practice

MEDIATORS
of
INFLAMMATION

Journal of
Diabetes Research

Disease Markers

Journal of
Immunology Research

International Journal of
Endocrinology

PPAR Research

BioMed
Research International

Hindawi

Submit your manuscripts at
http://www.hindawi.com

Journal of
Ophthalmology

Stem Cells
International

Evidence-Based
Complementary and
Alternative Medicine

Journal of
Obesity

Journal of
Oncology

Computational and
Mathematical Methods
in Medicine

Behavioural
Neurology

Parkinson's
Disease

AIDS
Research and Treatment

Oxidative Medicine and
Cellular Longevity