Subgingival periodontal pathogens in Down syndrome children without periodontal breakdown. A case–control study on deciduous teeth

C. Vocale[1], M. Montevecchi[2], G. D'Alessandro[2], M. Gatto[2], G. Piana[2], L.Nibali[3], M. C. Re[4], V. Sambri[5]

[1]*Unit of Microbiology, Regional Reference Centre for Microbiological Emergencies (CRREM), St. Orsola Malpighi Hospital, Bologna, Italy*
[2]*Department of Biomedical and Neuromotor Sciences (DIBINEM), Unit of Dental Care for Patients with Special Needs and Pediatric Dentistry, University of Bologna, Bologna, Italy*
[3]*Periodontology Unit, Centre for Host-Microbiome Interactions, Faculty of Dentistry and Oral Craniofacial Sciences, King's College London, London, United Kingdom*
[4]*Unit of Microbiology, Department of Medical and Surgical Sciences, "Alma Mater Studiorum" University of Bologna, S. Orsola-Malpighi Hospital, Bologna, Italy*
[5]*Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy*

e-mail: m.montevecchi@unibo.it

DOI 10.23804/ejpd.2021.22.04.9

Abstract

Aim Down syndrome is the most common form of aneuploidia compatible with a long survival. The affected subjects are more susceptible to severe early-onset periodontal disease and show a lower risk to develop dental caries than the non-affected population. This study investigated the prevalence of periodontal pathogens in the subgingival plaque of deciduous teeth in children with Down syndrome without signs of periodontal breakdown.

Methods Thirty children suffering from Down syndrome and 46 matched healthy subjects were studied. A total of 228 subgingival plaque samples from deciduous teeth were separately collected and evaluated by polymerase chain reaction assays.

Results The prevalence of Treponema denticola, Porphyromonas gingivalis, Prevotella intermedia, Aggregatibacter actinomycetemcomitans and Tannerella forsythia was investigated. Aggregatibacter actinomycetemcomitans and Tannerella forsythia were significantly more prevalent in Down syndrome children (respectively 8 and 9 times) than in controls.

Conclusion In absence of periodontal impairment, Down syndrome children display a clear presence of periodontal pathogens already in the deciduous dentition. The hypothesis of an intrinsic predisposing condition is here supported.

KEYWORDS Periodontal diseases; Down syndrome; Microbiology; Child; Tooth, deciduous.

Introduction

Down syndrome (DS) is the most common human aneuploidia compatible with prolonged life expectancy. The underlining genetic disorder is characterised by the presence of an extra copy of the chromosome 21. Patients affected by DS are more susceptible to early onset and severe forms of periodontal disease [Jepsen et al., 2018], while presenting a lower risk of dental caries than healthy subjects in the same age group [Castilho and Marta, 2010]. It has been hypothesised that the precocious nature and rapid progression of the periodontal disease may be due to the impaired immune response, the fragile periodontal tissue, or the early senescence typical of DS [Amano et al., 2001; Jepsen et., 2018]. However, the cause of periodontal disease tendency is not yet fully understood. In general, the onset and progression of periodontitis are clearly correlated with presence and increased numbers of specific bacteria.

A recent work by Nòvoa et al. [2020] has investigated the subgingival microbiome of patients with DS and periodontitis, comparing it with the microbiome of patients with DS without periodontitis. The results show significant differences between the oral microbiome at various taxonomical levels, and this finding is in agreement with those of previous studies on the subgingival microbiota and microbiome in the general population.

Several reports describing the prevalence of periodontal pathogens in young DS subjects have suggested an early colonisation of the oral cavity [Morisaki and Hamada 2000; Sakellari et al., 2001; Amano et al., 2001; Morinushi et al., 1997; Naka et al., 2009, Amano et al., 2000]. However, the available data are still scattered and insufficient to determine it. Generally, the detection of periodontal pathogens has been accomplished by cultural techniques, which are not usually sensitive enough and require special skill, especially in the isolation of anaerobic bacteria. Recently, improved methods, such as immunoassays using specific antibodies and DNA probe techniques, allowing specific and sensitive detections of periodontal and other pathogens, have been developed. However, these new methods still require approximately 103–105 targets per sample specimen. The use of polymerase chain reaction (PCR) can lower the detection limit to 102 cells per specimen. Nevertheless,

there have been few reports describing the subgingival bacterial prevalence in a DS population using this method. And even fewer studies focused on deciduous dentition, an aspect that could be relevant in order to understand the very early colonisation of the oral cavity of DS subjects.

The aim of this study was to investigate by PCR assay the prevalence of 5 putative periodontal pathogens in the subgingival environment of deciduous teeth in DS children without periodontal breakdown, and to compare the results with a matched healthy population in primary dentition.

Methods

Study population

The patients were recruited through the Dental Service for Patients with Special Needs and Pediatric Dentistry, Department of Biomedical and Neuromotor Science, University of Bologna. Two groups were created on the basis of presence (study group) or absence (control group) of DS syndrome. From consecutive patients, the study sample was identified by using the following inclusion criteria: developmental age, absence of clinically detectable periodontal breakdown, presence of at least a deciduous molar, an upper and a lower jaw deciduous incisor, and genetic DS diagnosis only for the study group.

Exclusion criteria were: non collaborating subjects, genetic and mental disorders (other than DS for the study group), systemic diseases or drugs intake that clearly affect periodontal condition or immune response in general, oral hygiene training and/or orthopaedic-orthodontic treatment and/or antibiotic therapy within the previous three months.

Informed consent was obtained from the legal tutor of each subject enrolled in the study. The research Ethics Committee of the Sant'Orsola-Malpighi Hospital of Bologna, Italy, approved the study (PG. N. 0019293 20 of June 2014).

Periodontal examination

The selected deciduous teeth were a first molar, an upper and a lower central incisor, Independently from the dental arch examined. If there were no deciduous first molars or central incisors, the adjacent primary tooth was selected. The mesial aspect of these teeth was investigated both microbiologically and clinically. After the microbiological collection, by means of a periodontal probe (CP-12 UNC SE, Hu-Friedy, Chicago, IL) a single experienced periodontist recorded the probing depth to the nearest millimetre (PD) and the bleeding on probing (BOP) [Guerrero et al., 2005] of each site. The full mouth plaque index (PI) [O'Leary et al., 1972] and the full mouth gingival index (GI) [Löe and Silness, 1963] were also recorded.

Preparatory to study, the examiner underwent calibration for clinical parameters.

Microbiological examination

The same operator involved in clinical recording collected the subgingival biofilm samples. Any antiseptic mouth-rinse was avoided before sampling. Technically, the selected site was isolated from saliva using cotton rolls and gently air dried; then, supragingival plaque was carefully removed using a Gracey's curette. A sterile paper point was inserted as deep as possible into the sulcus and left for 10s. All collected paper points were separately suspended in 1ml of sterile phosphate-buffered saline (pH=7.4) and then sent to the laboratory for processing within 24h. The bacterial examination was performed in blind respect to the tooth site and the sample groups. Bacteria genomic DNA was isolated with a DNA isolation automatic system NucliSENS easyMAG (Biomerieux, France) according to the manufacturer's instruction. Five species of periodontal pathogens, Treponema denticola (Td), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Aggregatibacter actinomycetemcomitans (Aa) and Tannerella forsythia (Tf), were identified using specific primers (Table 1). Universal primers that match almost all bacterial 16S rRNA genes were used as positive control. PCR amplification was performed using a Mastecycler gradient (Eppendorf); 10 ml of DNA template was added to a reaction mix containing: 10X Taq buffer, 10mM of each primer, 2mM of dNTPs, 25 mM of MgCl2 and 1U of Taq polymerase (Fermentas Life Science). Samples were preheated at 95 °C for 3 min and then amplified under the following conditions: denaturation at 95°C for 45 s and annealing at 58 °C for 45 s A total of 35 cycles were performed, until an elongation final step at 72 °C for 1 min was reached. The PCR products were subjected to electrophoresis in a 1% agarose gel that was stained with ethidium bromide and photographed under UV light.

Statistical analyses

With an 80% power and an α level of 0.05, hypothesising in DS subjects a 3 times higher chance of detection of each bacterium compared with controls, with a case control ratio of 1.5, at least 21 DS subjects and 32 controls were needed. Block matching was carried out. Frequency distribution and mean (standard deviation) described categorical or quantitative data. Given that PI and GI were not normally distributed (Shapiro-Wilks test p=0.001) median and interquartile range were used for description and Mann-Whitney U-test for the significance analysis. T-Student test and Chi-square test compared age gender between the two groups, respectively. Mixed effect model was used to analyse the influence of group (DS vs. Control) on frequency of detection of each bacterial species in the three sampling sites, considering group, BOP and PPD as fixed and site as random effect. Alfa level was set at 0.05.

Sets of PCR primers used in this study
T. denticola (316bp)*
5' TAA TAC CGA ATG TGC TCA TTT ACA T 3'
5' TCA AAG AAG CAT TCC CTC TTC TTC TTA 3'
P. gingivalis (404 bp)*
5' AGG CAG CTT GCC ATA CTG CG 3'
5' CTG TTA GCA ACT ACC GAT GT 3'
P .intermedia (256 bp)*
5' CGT GGA CCA AAG ATT CAT CGG T 3'
5' CTT TAC TCC CCA ACA AAA GCA 3'
T .forsythensis (746 bp)*
5' TAC AGG GGA ATA AAA TGA GAT ACG 3'
5' ACG TCA TCC CAA CCT TCC TC 3'
A. actinomycetemcomitans (285 bp)*
5' TCG CGA ATC AGC TCG CCG 3'
5' GCT TTG CAA GCT CCT CAC C 3'
** Expected size of PCR products*

TABLE 1 Sequence of PCR primers used.

Intraclass Correlation Coefficient (ICC) and its 95% Confidence Interval were used to evaluate the reliability of data. As for PD and BOP, reliability analysis was performed on 4 children with 20 teeth each, counting for 120 surfaces (6 sites per tooth). As for GI and PI, distal, medial, palatal and vestibular surfaces were examined on the same patients. Intra-observer reproducibility showed a good agreement for clinical parameters (ICC 0.88–0.69).

Results

A total of 30 collaborating children affected by DS (14 males, 16 females) with a mean age of 5.5 years (SD±1.2) and 46 controls (17 males, 29 females) with mean age of 4.5 years (SD±0.5) were enrolled in the study. Statistical difference for age was detected between the two groups (p=0.001).

The full mouth GI was 0.50 (0.50–0.87) for DS subjects and 0.25 (0-0.62) for the controls; the full mouth PI was 60% (50%-75%) for the study group and 69% (60%-80%) for controls. Statistical differences for both indexes were found between the two groups (PI p=0.012; GI p=0.007).

The microbiological and clinical investigations were performed on 228 deciduous teeth. In both samples the examined sites presented a median PPD of 1 mm (interquartile range=1). Bleeding on probing was positive for 20% and 15.2% of DS and control sites respectively (p=0.349). The data of the microbiological examination are reported in Table 2. The statistical evaluation did not show any significant differences between the two groups except for Tf and Aa (p=0.001).

Subjects with Trisomy 21 have a risk about 8 times higher to be positive for Aa and 9 times higher to be positive for Tf in comparison with controls (p=0.001). No significant influence of PPD and BOP on detection frequency was observed by means of multilevel analysis.

Discussion

The two groups investigated resulted generally homogeneous, if not for age, PI and GI.

DS subjects typically display a delayed eruption of the deciduous and permanent dentitions when compared with the general population [Ondarza et al. 1997]. Referring this aspect to the study design, where deciduous teeth were strictly required, can help explaining the slight but significant younger age of the control subjects respect to the DS group.

Interestingly, DS group shows lower value of PI but higher value of GI with respect to controls, suggesting a particular tissue reactivity of the periodontal soft tissue to oral biofilm. This consideration is in line with recent biomolecular findings showing how the oxidative burst activity of peripheral monocytes and granulocytes is elevated in DS individuals [Khocht et al., 2014]. The results of the present study show that, in absence of periodontal breakdown, subgingival microbiological differences exist between children with and without DS. Among the pathogens studied, statistical differences were detected for Tf and Aa, where both bacteria resulted more prevalent in DS children. The hypothesis that periodontopathogens are acquired during childhood in DS patients is here sustained, suggesting colonisation already in primary dentition.

About Aa the present work concords with the prevalent scientific line recognising a direct association between Aa and DS in young subjects [Sakellari et al., 2001; Morinushi et al., 1997; Sreedevi and Munshi 2000; Amano et al., 2000]. A limited number of studies have examined the composition of microbial plaque in DS children presenting pocket deep less than 3 mm [Sakellari et al., 2001; Amano et al., 2001; Amano et al., 2000].

Evaluating the correlation between the Aa isolation and the neutrophil chemotactic activity between DS and non-DS children without clinical attachment loss, Sreedevi and Munshi [1998] observed a strong association between the occurrence of Aa and the syndrome. In a subsequent study, comparing by PCR the bacterial prevalence in subgingival plaque samples between DS and age-matched controls children, Amano et al. [2000] partially confirmed the high rates of Aa for young DS subjects. In this study the population was divided into 4 groups by age with respect to dental status: 2 to 4 years, 5 to 7 years, 8 to 10 years and 11 to 13 years. The first two groups characterised by a primary dentition, and the remaining by a mixed dentition. The final outcome displayed a significant higher rate of Aa for DS subject only for the older group.

More recently, in a study ever based on a sensitive PCR assay, Sakellari et al. [2001] compared the subgingival microflora of children (8–13 year-olds), adolescents (13–18 year-olds) and young adults (18–28 year olds) to those of healthy individuals and subjects with cerebral palsy.. Also this study identifies a significant correlation with Aa and DS, but again only for adolescents and young adults.

About the Tf finding in the present study, results are consistent with those of Amano et al, where the pathogen was significantly more represented in DS subjects already from early childhood [Amano et al., 2000]. In contrast, in the study by Sakellari et al. [2001], Tf displayed a similar feature to Aa, showing a correlation to DS only from adolescence on.

Differently from the present paper, both studies found significant correlations between DS and Pg. While, with regard to the other two bacteria herein investigated, Td correlation with DS was positive for Amano et al. [2000], and Pi for Sakelari et al. [2001] respectively. The Pg and Td lower levels herein detected with respect to previous studies cannot have

	Exponential coefficient	95% confidence interval	P=	Observed positivity % D	ND
Aa	8.394	3.519-20.02	0.001	64	24
Td	1.741	0.431-7.026	0.435	3	0
Tf	9.194	3.923-21.548	0.001	51	12
Pg	1.345	0.350-5.024	0.658	3	1
Pi	1.606	0.742-3.473	0.228	28	19

TABLE 2 Influence of Down syndrome on detection frequency of studied bacteria accounting for site: multilevel analysis (Reference category: no Down).

a clear explanation; certainly the general good periodontal state of health can have played a role on it. It is actually known how probing depth and bleeding on probing can significantly influence the pathogens bacterial load [Gatto et al., 2014].

It must be said that, in spite of the apparent similarities, comparing microbiological results from different papers is not an easy task. Many are the variables conditioning the final results and even minor aspects can play a significant role in the final outcomes [Nguyen-Hieu, 2013]. In our opinion, the most relevant difference between our work and the two mentioned above is about teeth selection. Both studies were mainly oriented on permanent teeth and only when not present, they turned to deciduous ones. On the opposite, the present work was strictly focused on deciduous teeth.

This decision was made for two reasons; the first one derives from the assumption that time of tooth permanence in the oral cavity may affect the microbial composition. This has been recently confirmed by a microbiological study investigating and comparing the core microbiome between permanent and deciduous teeth [Shi et al., 2016]. Studying 20 individuals in mixed-dentition stage, Shi et al. [2016] suggested that microbial diversity exists among permanent and deciduous tooth sites. Interestingly, this study reports that the microbial communities of sites in deciduous teeth are more similar than those in permanent teeth. A possible explanation is that compared with the newly-erupted permanent teeth, the microbial communities on deciduous teeth have had greater opportunity for development and maturation. Previous studies have reported temporal shifts in the microbial communities of single teeth and pooled plaque [Brailsford et al., 2005; Takeshita et al., 2015]. Therefore, in a rapidly evolving dentition typical of childhood, the deciduous teeth may be considered the most reliable for microbial investigations.

The second reason is connected to the frequent tooth eruption disturbance of DS subjects [Ondarza et al., 1997]. Since our intention was to focus on early childhood, but at the same time a certain clinical collaboration of the subjects was required, the age-matched control group could have been in a mixed dentition already. Consequently, the specific choice of deciduous teeth as target for the microbiological sampling appeared us as the best option for a linear comparison. As previously said, this ***a priori*** setting on deciduous teeth is certainly the primary explanation of the age difference between the groups. A limit that is compensated by the microbiological advantages deriving form this choice. In our opinion, the delayed eruption associated to DS raises some concerns about the study design of Amano et al. [2000], where the study population was divided by age with respect to dental status. For instance, while it is quite common that a 5–7 year old DS child would display no permanent teeth, on the contrary a same age healthy subject would probably already be in a mixed dentition. It is consequently quite hard to identify the matching as declared by the authors if not under strictly, but not mentioned, inclusion criteria.

As a general finding, in spite of the overall differences among the few studies focused on DS children, all come to identify microbiological differences in respect to matched non-DS subject, and in particular an early colonisation by some of the main periodontal pathogens. The present study agrees with this hypothesis showing that microbiological differences are present already form the primary dentition.

In regard to the periodontal pathogens and the control group, the present study obtained a non-negligible percentage of detection for Aa (20%). This observation is in line with previous findings considering the detection of this pathogen quite common in periodontally healthy young subjects [Yang et al., 2002; Riep et al., 2009]. Nevertheless, in many studies Aa was found to be characteristically associated with the aggressive form of periodontitis in systemically healthy young subjects, where the prevalence of this pathogen displays a clear surge [Christersson 1993; Darveau et al., 1997; Shaddox et al., 2012].

As demonstrated in prospective cohort studies in specific populations, the presence of Aa in periodontally healthy children could represent a risk marker for localised aggressive periodontitis initiation [Haubek et al., 2008]. It is not clear whether a similar pattern of periodontal disease onset and progression could be associated also with this DS population. Longitudinal evaluation of this population could lead to important understanding of the effective role of these bacteria in the initiation and progression of periodontitis.

The results here presented corroborate the hypothesis that the putative periodontal pathogens belong to the early commensal microbiota in children with DS, and that their presence at high levels may represent a predisposition to periodontal disease. Additional information may be obtained by using an ecological approach to investigate the distribution of the microbial consortia in the subgingival environment of children with DS. We are indeed aware that the current periodontal microbiological paradigm suggests that further studies should be based on open-ended molecular techniques, able to allow the identification of a much broader spectrum of pathogenic species.

In summary, despite the similarities between the evaluated subgingival microbial composition of children with and without DS, individuals with DS showed a higher level of Aa and Tf. These findings are consistent with the notion that specific subgingival bacteria may contribute to the increased risk of development of periodontal disease.

A line of research suggests that severe periodontal destruction observed among DS subjects is due to various factors mainly related to their chromosomal abnormality [Reuland-Bosma et al., 2001; Chaushu et al., 2002]. However, the issue of whether altered host response allows for easier colonisation and proliferation of periodontal pathogens, as reported in the present study, remains unclear. Despite that, the important role of controlling microbial plaque in subjects with DS is crucial, starting already from the primary dentition. Beginning from an increasing awareness of their parents/guardians, preventive efforts should therefore focus on regular periodontal monitoring and on preventing changes about those parameters responsible for the pathogenic bacteria overgrowth, including oral hygiene, antiseptics, probiotics, diet and host-related factors.

Conclusion

The present study shows that even in absence of periodontal impairment, DS children display the presence of periodontal pathogens already in deciduous dentition. A specific effort in preventive dentistry seems to be justified since the very early age for these patients. Further studies on broader samples and larger bacterial ranges are anyway suggested.

Acknowledgements

The authors declare that there are no conflicts of interest

and sources of funding with respect to the authorship and/or publication of this article.

Conflict of interest statement

The authors declare the absence of any potential conflict of interests.

References

- Amano A, Kishima T, Akiyama S, Nakagawa I, Hamada S, Morisaki I. Relationship of periodontopathic bacteria with early-onset periodontitis in Down's syndrome. J Periodontol 2001;72(3):368-73.
- Amano A, Kishima T, Kimura S, Takiguchi M, Ooshima T, Hamada S, Morisaki I. Periodontopathic bacteria in children with Down syndrome. J Periodontol 2000;71(2):249-55.
- Brailsford SR, Sheehy EC, Gilbert SC, et al. The microflora of the erupting first permanent molar. Caries Res 2005;39(1):78-84.
- Castilho AR, Marta SN. Evaluation of the incidence of dental caries with Down syndrome after their insertion in a preventive program. Cien Saude Colet 2010;15(Suppl 2):3249-53.
- Chaushu S, Yefenof E, Becker A, Shapira J, Chaushu G. Severe impairment of secretory Ig production in parotid saliva of Down Syndrome individuals. J Dent Res 2002;81(5):308-12.
- Christersson LA. Actinobacillus actinomycetemcomitans and localized juvenile periodontitis. Clinical, microbiologic and histologic studies. Swed Dent J Suppl 1993;90:1-46.
- Darveau RP, Tanner A, Page RC. The microbial challenge in periodontitis. Periodontol 2000 1997;14:12-32.
- Gatto MR, Montevecchi M, Paolucci M, Landini MP, Checchi L. Prevalence of six periodontal pathogens in subgingival samples of Italian patients with chronic periodontitis. New Microbiol 2014;37(4):517-24.
- Guerrero A, Griffiths GS, Nibali L et al. Adjunctive benefits of systemic amoxicillin and metronidazole in non-surgical treatment of generalized aggressive periodontitis: a randomized placebo-controlled clinical trial. J Clin Periodontol 2005;32(10):1096-107.
- Khocht A, Russell B, Cannon JG, Turner B, Janal M. Oxidative burst intensity of peripheral phagocytic cells and periodontitis in Down syndrome. J Periodontal Res 2014;49(1):29-35.
- Jepsen S, Caton JG, Albandar JM, Bissada NF, Bouchard P, Cortellini P, Demirel K, de Sanctis, M, Ercoli C, Fan J, et al. Periodontal manifestations of systemic diseases and developmental and acquired conditions: Consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol 2018;20:219-29.
- Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:533-51.
- Morinushi T, Lopatin DE, Van Poperin N. The relationship between gingivitis and the serum antibodies to the microbiota associated with periodontal disease in children with Down's syndrome. J Periodontol 1997;68(7):626-31.
- Morisaki I, Hamada S. Periodontopathic bacteria in children with Down syndrome. J Periodontol 2000;71(2):249-55.
- Naka S, Yamana A, Nakano K, et al. Distribution of periodontopathic bacterial species in Japanese children with developmental disabilities. BMC Oral Health 2009;23;9:24.
- Nguyen-Hieu T. Microbial sampling process can change results of microbiological analysis in periodontitis diagnosis. A minireview. J Investig Clin Dent 2013;4(3):151-9.
- Nóvoa L, Sánchez MDC, Blanco J, Limeres J, Cuenca M, Marín MJ, Sanz M, Herrera D, Diz P. The subgingival microbiome in patients with down syndrome and periodontitis.
- J Clin Med. 2020 Aug; 9(8): 2482. Published online 2020 Aug 2. doi: 10.3390/jcm9082482.
- O'Leary TJ, Drake RB, Naylor JE. The plaque control record. J Periodontol 1972;43(1):38.
- Ondarza A, Jara L, Muñoz P, Blanco R. Sequence of eruption of deciduous dentition in a Chilean sample with Down's syndrome. Arch Oral Biol 1997;42(5):401-6.
- Reuland-Bosma W, van der Reijden WA, van Winkelhoff AJ. Absence of a specific subgingival microflora in adults with Down's syndrome. J Clin Periodontol 2001;28(11):1004-9.
- Riep B, Edesi-Neuss L, Claessen F, et al. Are putative periodontal pathogens reliable diagnostic markers? J Clin Microbiol 2009;47(6):1705-11.
- Sakellari D, Belibasakis G, Chadjipadelis T, Arapostathis K, Konstantinidis A. Supragingival and subgingival microbiota of adult patients with Down's syndrome. Changes after periodontal treatment. Oral Microbiol Immunol 2001;16(6):376-82.
- Shaddox LM, Huang H, Lin T et al. Microbiological characterization in children with aggressive periodontitis J Dent Res 2012;91(10):927-33.
- Shi W, Qin M, Chen F, Xia B. Supragingival microbial profiles of permanent and deciduous teeth in children with mixed dentition. PLoS One 2016; 11(1):e0146938.
- Sreedevi H, Munshi AK. Neutrophil chemotaxis in Down syndrome and normal children to Actinobacillus actinomycetemcomitans. J Clin Pediatr Dent 1998;22(2):141-6.
- Takeshita T, Yasui M, Shibata Y, et al. Dental plaque development on a hydroxyapatite disk in young adults observed by using a barcoded pyrosequencing approach. Sci Rep 2015;5:8136.
- Yang EY, Tanner AC, Milgrom P, et al. Periodontal pathogen detection in gingiva/tooth and tongue flora samples from 18- to 48-month-old children and periodontal status of their mothers. Oral Microbiol Immunol 2002;17(1):55-9.