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Periodontal and microbiological parameters in children and adolescents with cleft lip and/or palate

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Objective. To evaluate the oral hygiene and the periodontal condition of children and adolescents with cleft lip and/or palate (CLP).

Methods. Forty-one children and adolescents, 4–18 years, with CLP and 41 normal controls participated. Clinical parameters examined were the plaque and gingival index and Community Periodontal Index of Treatment Needs. For teeth in the cleft area, probing pocket depth, bleeding on probing, and tooth mobility were also evaluated. Samples of subgingival plaque were collected from 21 randomly selected patients of each group.

Results. The CLP group had generally poorer oral hygiene (plaque index significantly higher) compared with the control. Children in both groups presented mild degree of gingivitis. Teeth in the cleft area had significantly higher pocket probing depth and tooth mobility, compared with corresponding teeth in the control group. The microbial analysis did not reveal significant differences in the composition of the subgingival microbiota between groups. Teeth in the cleft presented higher isolation frequencies and mean percentages of periodontopathic bacteria.

Conclusion. Youngsters with CLP showed poor oral hygiene and worse periodontal condition, compared with controls. The above results advocate their participation in an intensive preventive dental programme that should start at an early age, possibly decreasing the risk of future periodontal disease.

Introduction

The cleft of the lip, alveolus and/or palate is the most common congenital malformation of the face. The prevalence of this anatomic defect varies with such factors as race, geographic location, sex, and type of defect. The estimated mean prevalence in Greece is 0.80–1.12 in 1000 live births[1]. As a result of the fact that children with cleft lip and/or palate (CLP) experience aesthetic, speech, hearing, dental, and even psychological problems, their treatment dictates a multidisciplinary approach from different health disciplines.

Children and adolescents with CLP are considered to be at increased risk for the development of carious and periodontal diseases, which are related both because of the anatomical defect as well as the long-term surgical, orthodontic, and prosthodontic therapeutic interventions[2]. The cleft deformity, the soft tissue folds, the shallow vestibule, the dental arch irregularities, the long-term orthodontic treatment and even the scar tissue observed in the region after surgical closure of the cleft defect hinder optimal oral hygiene control[3]. The periodontal health in the cleft region is further deteriorated because of the poorly developed osseous structures, in the cases of osseous clefts, as well as the iatrogenic trauma caused to the periodontium by the orthodontic therapy and the sub-gingival margins of prosthetic restorations[4,5]. Considering all the above factors, the question arises as to whether subgingival microbiota of the cleft area may favour the growth of putative periodontopathic pathogens[2,6].

Few studies in the current literature have addressed the periodontal health in children and adolescents with CLP[3,7–12]. Furthermore,

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only three studies have examined the microbial composition of subgingival plaque of teeth in the cleft area and only one of them was carried out in children with primary dentition[2,6,13].

The purpose of this study was to evaluate the oral hygiene status, the periodontal condition and the composition of the subgingival microbiota of children and adolescents with CLP and to compare the above parameters with those of normal noncleft children and adolescents.

Material and methods

Eighty-two children and adolescents, 4–18 years old, with a primary, mixed or permanent dentition, patients of the postgraduate clinic of the Pediatric Dentistry Department of the Dental School of the University of Athens, were included in this study. From these, forty-one subjects had surgically repaired unilateral or bilateral CLP and comprised the test group. The remaining 41 normal noncleft children and adolescents comprised the control group and were matched for sex, age, and orthodontic treatment status (Table 1).

The study subjects had free medical history. Information regarding the use of medicines from the child or the mother during pregnancy as well as the type and timing of reconstructive surgeries (in the case of children and adolescents with cleft) were obtained. Ethical approval was received from the Committee for Ethics and Research of the Athens Dental School, in accordance with the Helsinki Declaration.

Periodontal examination

The dental and periodontal examination for all subjects was performed by one of the authors (HP), using a dental mirror and a WHO periodontal probe. The plaque index (PlI)[14] and the gingival index (GI)[15] were measured for each of the six Ramfjord teeth (16, 12, 24, 36, 32, and 44). In the cases of primary or mixed dentition the second primary molar was recorded instead of the first permanent molar and the first primary molar instead of the first permanent molar. Furthermore, the plaque and gingival indices were recorded in the six upper anterior in both groups of children.

The periodontal disease status of each subject was evaluated with the aid of the Community Periodontal Index of Treatment Needs (CPITN),[16] and the probing pocket depth (PPD) of the teeth in the cleft area was recorded. Specifically in children and adolescents with cleft, the measurements were performed on all surfaces of the two teeth neighbouring the cleft and the tooth in the cleft area in cases that this was present. Accordingly in the control group, the measurements of the PPD were performed in the upper central and lateral incisors and upper canines (UC). Moreover, bleeding following mild probing was registered in the above teeth (where PPD was measured) and was classified as present or absent. Finally, dental mobility in the same teeth was verified manually and scored as follows: 0: representing physiologic mobility, 1: mobility of the tooth in a horizontal direction up to 1 mm, 2: mobility of the tooth in a horizontal direction up to 2 mm, and 3: mobility of the tooth in both horizontal and vertical direction up to 3 mm.

Composition of subgingival microbiota

Sampling procedure. From the 41 subjects in the study, 21 were randomly selected for the evaluation of their microbiological profile. Children with a primary dentition were not

Table 1. Distribution of children and adolescents in cleft and control groups according to gender, age, and orthodontic status.

	Cleft group		Control group	
	Boys *n* (age ± SD)	Girls *n* (age ± SD)	Boys *n* (age ± SD)	Girls *n* (age ± SD)
No orthodontic treatment	4 (6.9 ± 2.5)	6 (7.6 ± 2.5)	4 (7.3 ± 2.5)	6 (8.7 ± 1.7)
Orthodontic treatment	19 (11.2 ± 2.5)	12 (12.3 ± 3.8)	19 (11.5 ± 2.1)	12 (11.7 ± 3.9)
Totals	23 (10.4 ± 2.9)	18 (10.6 ± 3.9)	23 (10.7 ± 2.7)	18 (10.7 ± 3.6)

sampled. Following the evaluation of the PlI, three pooled samples of subgingival plaque were obtained from pre-selected sites. More precisely, one sample was obtained from the mesial surfaces of the teeth neighbouring the cleft and one sample from the tooth in the cleft (mesial and distal surfaces), in cases that there was one present. The third sample was obtained from the mesial surfaces of the first permanent molars (or the primary second molars, in case the first permanent molars had not yet erupted). Accordingly in the control group, two pooled samples of subgingival plaque were collected from 21 children that were again matched to the subjects of the test group for sex, age, and stage of orthodontic treatment. One sample was from the upper anterior teeth (mesial surface of 13 or 53 and distal surface of 11 or 51) and one sample from the mesial surfaces of the first permanent molars (or the primary second molars). Plaque sample collection was performed by placing two fine paper-points into each pocket comprising the interdental area. They were left in place for 10 s and then removed.

The samples were placed in 0.9 mL of pre-reduced anaerobically sterilised transport fluid (RTF) and transferred to the microbiological laboratory within 10 min. The samples were subsequently dispersed for 10 s on a Vortex mixer and 10-fold serially diluted in RTF, to be cultured and examined by dark field.

Microbiological techniques. Dark field microscopy: For analysis by dark field microscopy, 0.1 mL from the initial suspension was removed and placed on a glass slide. The morphological evaluation of the bacterial cells as well as their motility were performed at a magnification of 1250×.

Culturing: Aliquots of 0.1 mL of the appropriate dilutions were plated in duplicate onto Enriched Trypticase Soy Agar (ETSA) supplemented with 4% defibrinated human blood[17] to determine the composition of the predominant cultivable microbiota and onto TSBV [trypticase-soy-agar (BBL Microbiology systems, Cockeysville, MD, USA) supplemented with 5% serum 75 lg/mL bacitracin and 5 lg/mL vancomycin] to isolate *Aggregatibacter actinomycetemcomitans*[18].

The ETSA plates were incubated at 37°C for 5 days anaerobically in a jar using a gaspack (Anaerogen, Oxoid, Hampshire, UK) (5% CO_2 + 10% H_2 + 85% N_2) and the second set of ETSA plates remained in the jar for another 7 days for the detection of black-pigmenting as well as the slow growing colonies, which might be isolated at low dilutions.

The TSBV plates were incubated at 37°C in air plus 10% CO_2 in a jar using a Gaspack CO_2 generating sachet (Becton Dickinson, Microbiology Systems, Cockeysville, MD, USA) for 5 days. Following incubation, optimum dilution ETSA plates giving 40–250 colonies were selected and the total number of colonies was counted. Therefore, total counts (Colony Forming Units – CFU – per mL), both anaerobic and facultative, were determined on ETSA plates. From the 5-day incubated ETSA plates, subcultures of five similar colonies of every colonial type found under the stereo-microscope were made on blood agar plates [40 g/L blood agar base (Difco Laboratories, Detroit, MI, USA) supplemented with 7% defibrinated blood]. The blood agar plates were incubated anaerobically and in air plus 10% CO_2 at 37°C for 2 days to check the purity of the isolates and to determine their atmospheric requirements.

Characterisation and identification: Characterisation and identification was performed to genus and species level based upon colony and cellular morphology, Gram stain reaction, motility, respiration requirements, and biochemical reactions using micromethod kit system (API 20 STREP, API 20A). Additional enzymatic activities were tested with the aid of API ZYM (API System, Verdieu, France). Minitek anaerobe systems (BBL Microbiology systems, Cockeysville, MD, USA) including the following tests were also used: acid production from dextrose, maltose, lactose, sucrose, sorbitol, inositol, cellobiose, and ornithine, as well as the production of H_2S and urease.

Statistical methods

The statistical analysis of the data was performed using the statistical package of STATA corporation (College Station, TX, USA).

The *t*-test was used to test for differences in the mean values of a particular variable between the group of cleft lip/palate patients and the control group. The hypothesis of the equality of means between any two variables within each group was tested using the unpaired *t*-test. Frequency tables were also calculated, whereas the existence of association was measured using Pearson chi-squared test and Fisher's exact test. All hypotheses were tested using the 95% probability.

Results

Clinical results

From the cleft lip/palate patients, five had isolated cleft palate, 26 unilateral, and 10 bilateral cleft lip and palate (Table 2). All study subjects with a cleft had significantly greater mean PlI scores ($P < 0.005$) than the control children (Table 3). The highest PlI was found in both groups of children and adolescents in the upper front teeth (test group 1.16 and control group 0.82), whereas the Ramfjord elements had slightly lower mean plaque scores (1.03 for test and 0.76 for control). The children with cleft, however, showed a tendency for increase in the mean PlI up to the age of 16. At the ages of 17 and 18 years (for the three subjects in each group) the level of oral hygiene improved, but the cleft subjects continued to show the highest score (Table 3).

Table 2. Mean probing depth of teeth neighbouring the cleft and teeth in the cleft in the test group, according to the type of cleft defect.

	UCLP (*n* = 26)	BCLP (*n* = 10)	CP (*n* = 5)
IC			
n = 14	2.23 ± 0.55	2.2 ± 0.42	–
NC			
n = 41	2.19 ± 0.49	2.14 ± 0.38	2.08 ± 0.30
Gender			
Boys	10	4	4
Girls	16	6	1

Values are given as mm ± SD.
UCLP, unilateral cleft of the lip, alveolus, and palate; BCLP, bilateral cleft of the lip, alveolus, and palate; CP, isolated cleft of the palate; NC, neighbouring the cleft; IC, in the cleft.
n, number of children; SD, standard deviation.
Gender distribution per type of cleft defect is also presented.

Table 3. Mean Plaque Index and Gingival Index of the Ramfjord elements and upper anterior teeth according to age groups.

	PII Cleft	PII Normal	GI Cleft	GI Normal
A. Ramfjord				
4–5 (*n* = 2/group)	0.75	1.08	0.70	0.65
6–12 (*n* = 27/group)	1.04	0.86	1.05	1.01
13–16 (*n* = 10/group)	1.12	0.71	1.28	1.02
17 & 18 (*n* = 3/group)	0.88	0.55	0.98	1.09
Mean	1.03	0.76	1.08	1.01
B. Upper Anterior				
4–5	1.00	1.40	0.87	0.50
6–12	1.17	0.83	1.10	1.02
13–16	1.11	0.82	1.46	1.06
17 & 18	0.92	0.51	0.79	1.12
Mean	1.16	0.82	1.23	1.03

PII, Plaque Index; GI, Gingival Index.

A mild degree of gingival inflammation was noted in all children (Table 3). No significant differences were found in the mean GI, between subjects with cleft and those of the control group, both in the Ramfjord teeth (1.08 and 1.01, respectively) as well as the upper front teeth (1.23 and 1.03, respectively). Here, too a tendency for increase in inflammation for the subjects with cleft can be noted up to the age of 17 years.

According to the CPITN index there was no code 0 classification for children and adolescents in the test group, while 10% of those in the control group presented with healthy periodontal tissues (code 0). Code 1, involving bleeding on probing, was found in 0% of the children in the test group and in 7% of the control group. A notable finding is that the majority of subjects in both groups (68% of the test group and 78% of the control group), presented with calculus (code 2), mostly in the lingual surfaces of the lower anterior teeth. Code 3, corresponding to pocket probing depths of up to 5.5 mm, were found more frequently in children of the test group (31%) compared with the control group (5%). Periodontal pocket depth more than 5.5 mm was not found in any patient (Fig. 1).

There were no statistical significant differences in the mean probing depth of teeth neighbouring the cleft (NC) and teeth in this area (IC) (Table 2). All children and

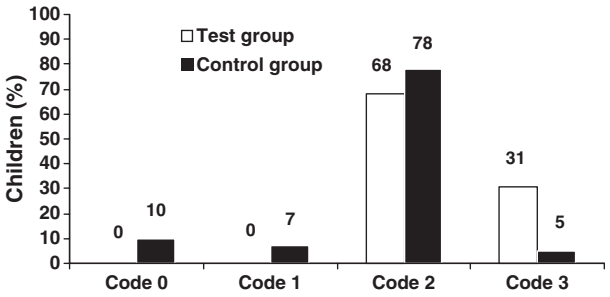

Fig. 1. CPITN index in children and adolescents of the control and the test group. Code 0 = healthy periodontal tissue, Code 1 = bleeding observed after probing with no calculus, Code 2 = calculus detected during probing or directly, Code 3 = pocket probing depth 3.5–5.5 mm, and Code 4 = pocket probing depth 6 mm or more.

adolescents with cleft presented mean probing depth 2.08–2.23 mm in the upper front teeth (NC and IC teeth). There was no statistically significant difference between different types of clefts.

By contrast, there was a significant difference in the probing depth of the upper front teeth between the test and the control group ($P < 0.05$). Teeth NC and teeth IC, in the test group, presented greater probing depth compared with the corresponding teeth of the control group (Table 4).

Teeth NC in the test group, exhibited statistically significant ($P < 0.05$) higher percentage of surfaces that bled (42%) on probing compared with the upper incisors (UI) of the control group (29%). There were no significant differences in the bleeding on probing between teeth IC (25%) and UI (29%) and UC (30%) of the control group.

None of the examined teeth in the control group exhibited pathologic mobility. By contrast, teeth NC, and teeth IC in the test group, presented mobility (1–3 scores) in high percentages (up to 65% of the examined teeth) depending on the type of cleft defect. Children with bilateral cleft of the lip, presented the higher percentage of teeth (20%) with score 3 mobility (Table 5).

Table 4. Probing depth of teeth neighbouring the cleft and teeth in the cleft in the test group, and upper incisors and upper canines in the control group.

Test group (n = 41)		Control group (n = 41)	
IC (n = 14)	2.22 ± 0.49	UI (n = 41)	1.85 ± 0.38
NC (n = 36)	2.14 ± 0.4	UC (n = 41)	1.69 ± 0.42

Values are given as mean (mm) ± SD.
Both tooth types in the test patients were found to have statistically greater sulci depths ($P < 0.007$) in comparison to both types in the control group. NC, neighbouring the cleft; IC, in the cleft; UI, upper incisors; UC, upper canines.

Microbiological results

The bacterial morphotypes obtained by dark field microscopic examination are presented in Table 6. Although the differences between the tooth types in the two groups of subjects remained relatively small, the teeth in the cleft area harboured significantly higher proportions of motile bacteria and spirochetes. Furthermore, the control group presented significantly higher proportion of motile bacteria in their molars, compared with the molars of the test group ($P < 0.05$).

Analysis of the subgingival microbiota showed great diversity, with 34 different bacterial species isolated. The number of facultative and anaerobic CFU/mL in subgingival plaque samples from the different sites are presented in Table 7. The total microbial count (CFU/mL) of upper incisors and canines and molars (UIC, M) of children and adolescents in the control group was significantly higher compared with the total count of those in the test group. Teeth in the cleft area (IC) and teeth neighbouring the cleft (NC), did not harbour more bacteria than the corresponding teeth of the control group.

The data presented in Table 8 summarise the prevalence (frequency of detection) of bacterial species in subjects with cleft and those in the control group. The most frequently isolated bacterial species in test group were: *Streptococcus* spp., *Veillonella* spp., *Actinomyces* spp., *Capnocytophaga* spp., *Fusobacterium* spp., *Prevotella intermedia/nigrescens*, and *Parvimonas micra* (previously *Peptostreptococcus micros*). Accordingly, in the control group the most frequently isolated bacterial species were: *Streptococcus* spp., *Veillonella* spp., *Actinomyces* spp., *Capnocytophaga* spp., *Fusobacterium* spp., *P. intermedia/nigrescens*, *P. micra*, *Gemella morbilorum*, and *Prevotella melaninogenica*.

Table 9 presents the relative proportions of bacterial species isolated in teeth of both

Table 5. Percentage (%) of teeth exhibiting mobility in subjects of the test group, according to the type of cleft defect and in children of the control group.

	NC tooth mobility (%) *n* = 36				IC tooth mobility (%) *n* = 14			
	Score 0	**Score 1**	**Score 2**	**Score 3**	**Score 0**	**Score 1**	**Score 2**	**Score 3**
Test group								
UCLP	33	56	0	11	65	27	8	0
BCLP	60	20	0	20	50	35	15	0
	UI tooth mobility (%) *n* = 41				**UC tooth mobility (%) *n* = 41**			
	Score 0	**Score 1**	**Score 2**	**Score 3**	**Score 0**	**Score 1**	**Score 2**	**Score 3**
Control group								
	100	0	0	0	100	0	0	0

Table 6. Proportion of different bacterial morphotypes observed by dark field microscopy in the subgingival plaque of teeth in the cleft, teeth neighbouring the cleft, molars of subjects with cleft, the upper incisors and canines of children in the control group, and molars of subjects in the control group.

	% Cocci (±SD)	**% Motiles (±SD)**	**% Spirochetes (±SD)**	**% Fusiform (±SD)**	**% Rods (±SD)**
IC (*n* = 8)	60.15 (–3.66)	**6.10*** (–1.5)	**1.4*** (–1.3)	8.38 (–1.48)	23.92 (–3.5)
NC (*n* = 21)	61.9 (–5.14)	**5.35*** (–1.62)	**1.18*** (–1.42)	7.35 (–1.79)	24.23 (–5.43)
MC (*n* = 21)	67.19 (–4.57)	**4.49**** (–1.45)	0.75 (–1.01)	6.73 (–1.62)	20.83 (–3.12)
UIC (*n* = 21)	67.74 (–5.02)	5.43 (–1.71)	0.74 (–1.03)	8.58 (–2.69)	17.51 (–3.72)
M (*n* = 21)	64.62 (–6.4)	**6.22**** (–2.14)	1.26 (–1.43)	9.47 (–3.38)	18.43 (–2.87)

*Statistically significant difference in upper incisors and canines $P < 0.05$.
**Statistically significant difference in molars $P < 0.05$.
IC, in the cleft; NC, neighbouring the cleft; MC, molars of subjects with cleft; UIC, upper incisors and canines of children in the control group; M, molars of subjects in the control group.

test and control groups. *Streptococccus* spp. constituted the most predominant species in all sites in all children and adolescents, but the relative proportion was significantly higher in the control group, compared with the same teeth in the test group. Furthermore, study subjects with cleft presented higher relative proportion of Gram-negative anaerobic rods in teeth in the cleft (IC) compared with the upper incisors and canines of the control group.

Discussion

The oral health of children and adolescents with CLP has been evaluated in the literature by examining mostly the presence of carious lesions, the oral hygiene level, and the degree of gingival inflammation[11–13,19]. The data on the periodontal health of children and adolescents with CLP are scarce and only two studies have evaluated the composition of the subgingival microbiota in children with complete cleft lip and palate[2,13]. Currently, there are no published data available on the oral health and the prevalence of periodontal disease in children and adolescents with CLP in Greece.

According to several studies, the oral health status and the presence of specific pathogenic microorganisms in subgingival plaque can be related to progressive periodontal destruction, and can influence the treatment outcome of the multidisciplinary team approach for clefts[2,6,11,13,20]. Therefore, this study is the first attempt to evaluate the periodontal condition and the composition of the subgingival microbiota of children and adolescents with CLP in Greece and to compare the above parameters with a control group of normal noncleft children matched for age, sex, and stage of orthodontic treatment. Great pains were taken to ensure that the control group was completely matched to the cleft patients, especially in relation to the presence (which

Table 7. Mean total bacterial counts of cultured bacterial species in test and control groups in the subgingival plaque of teeth in the cleft, teeth neighbouring the cleft, molars of children with cleft, the upper incisors and canines of children in the control group, and molars of children in the control group.

	Test group			Control group	
Bacterial species	**IC (*n* = 8)**	**NC (*n* = 21)**	**MC (*n* = 21)**	**UIC (*n* = 21)**	**M (*n* = 21)**
Gram-positive facultative anaerobic cocci	3.0×10^6	5.0×10^6	7.9×10^6**	1.5×10^8	8.7×10^7**
Gemella hemolysans	0	0	0	6.3×10^7	2.7×10^7
Streptococcus spp.	3.0×10^6	5.0×10^6	7.9×10^6	1.3×10^8	8.0×10^7
Gram-negative facultative anaerobic cocci	1.8×10^6	0	3.0×10^5	0	0
Neisseria spp.	1.8×10^6	0	3.0×10^5	0	0
Gram-positive anaerobic cocci	6.8×10^5	2.1×10^6	3.6×10^6	4.3×10^7	1.0×10^7
Gemella morbillorum	0	7.9×10^5	3.0×10^6	4.9×10^7	1.5×10^7
Parvimonas micra	4.9×10^5	1.6×10^6	4.8×10^6	1.9×10^7	3.8×10^6
Streptococcus intermedius	4.9×10^5	2.7×10^6	5.5×10^5	3.8×10^6	3.9×10^5
Gram-positive facultative anaerobic rods	2.1×10^6	3.3×10^6	5.2×10^6**	4.4×10^7	4.3×10^6**
Actinomyces spp.	1.7×10^6	2.2×10^6	4.3×10^6	4.4×10^7	4.3×10^7
Lactobacilli spp.	2.8×10^6	1.9×10^6	1.3×10^6	0	0
Rothia dentocariosa	0	1.7×10^6	2.7×10^6	0	0
Gram-positive anaerobic rods	9.3×10^5	8.2×10^5	8.5×10^5	1.7×10^7	9.9×10^6
Actinomyces israelii	4.8×10^5	1.0×10^6	5.9×10^5	7.7×10^6	6.7×10^6
Bifidobacterium spp.	1.5×10^6	7.5×10^5	7.7×10^5	2.4×10^7	2.7×10^5
Eubacterium spp.	2.5×10^5	3.3×10^5	5.1×10^5	1.2×10^7	1.3×10^6
Gram-negative anaerobic cocci	4.7×10^5	1.5×10^6	2.3×10^6	3.2×10^7	4.9×10^6
Veillonella spp.	4.7×10^5	1.5×10^6	2.3×10^6	3.2×10^7	4.9×10^6
Gram-negative facultative anaerobic rods	1.1×10^6	2.5×10^6	3.8×10^6**	4.3×10^7	3.0×10^7**
Capnocytophaga spp.	7.5×10^5	2.1×10^6	3.4×10^6	3.9×10^7	2.6×10^7
Eikenella corrodens	5.0×10^5	6.4×10^5	6.8×10^6	2.1×10^7	1.8×10^7
Haemophilus spp.	3.4×10^5	1.5×10^6	1.4×10^6	1.0×10^5	0
Gram-negative anaerobic rods	1.3×10^6	4.3×10^6	5.5×10^6**	4.9×10^7	4.0×10^7**
Bacteroides spp.	5.0×10^5	1.2×10^5	3.0×10^5	8.7×10^6	1.5×10^7
Bilophilla wadworthia	1.1×10^5	3.0×10^5	2.0×10^6	0	0
Campylobacter spp.	0	2.3×10^6	1.2×10^6	1.4×10^7	1.3×10^7
Wollinella spp.	1.1×10^5	3.5×10^6	4.9×10^6	0	0
Fusobacterium spp.	9.5×10^5	2.3×10^6	3.4×10^6	4.4×10^7	1.6×10^7
Porphyromonas gingivalis	4.6×10^5	3.9×10^5	3.0×10^6	1.3×10^6	2.1×10^7
Prevotella intermedia/nigrescens	3.2×10^5	8.6×10^5	2.6×10^6	6.4×10^6	2.0×10^7
Prevotella loeschii	2.7×10^5	1.8×10^6	7.8×10^5	4.2×10^7	1.2×10^7
Prevotella melaninogenica	0	0	0	1.7×10^6	1.4×10^7
Prevotella oralis	0	0	0	4.1×10^7	1.5×10^7
Selenomonas spp.	1.6×10^5	2.3×10^6	5.2×10^5	1.1×10^6	1.5×10^7
Bacterial species IF <12.5	6.9×10^5	2.8×10^6	3.3×10^6	0	0
Staphylococcus epidermidis	0	3.0×10^6	9.1×10^6	0	0
Stomatococcus spp.	5.0×10^5	5.0×10^5	1.9×10^5	0	0
Corynobacterium matrucotii	3.0×10^5	2.0×10^5	2.4×10^6	0	0
Clostridium spp.	1.2×10^6	1.9×10^6	6.8×10^5	0	0
Leptotrichia buccalis	6.0×10^4	1.0×10^5	1.0×10^5	0	0
Porphyromonas assaccharolytica	0	1.2×10^7	3.4×10^6	0	0
Propionibacterium spp.	0	6.6×10^4	1.8×10^6	0	0
Gram-positive total	6.0×10^6	1.1×10^7	1.6×10^7**	2.2×10^8	1.4×10^8**
Gram-negative total	2.8×10^6	8.3×10^6	1.1×10^7**	1.1×10^8	6.3×10^7**

Values are given as CFU/mL.

**$P < 0.005$.

IC, in the cleft; NC, neighbouring the cleft; MC, molars of subjects with cleft; UIC, upper incisors and canines of children in the control group; M, molars of subjects in the control group.

included the type) or absence of orthodontic appliances. This was true also for the patients that were sampled for culturing.

The oral hygiene level of control children and adolescents in our study was found to be fair (PlI = 0.76 ± 0.29) while that of children with cleft was moderate (PlI = 1.03 ± 0.35). There was a statistically significant difference between the two groups for the PlI, especially in the upper anterior area where a larger

Table 8. Isolation frequency of bacterial species in test and control groups in the subgingival plaque of teeth in the cleft, teeth neighbouring the cleft, molars of children with cleft, the upper incisors and canines of children in the control group, and molars of children in the control group.

	Test group			Control group	
Bacterial species	**IC (*n* = 8)**	**NC (*n* = 21)**	**MC (*n* = 21)**	**UIC (*n* = 21)**	**M (*n* = 21)**
Gram-positive facultative anaerobic cocci	100.0	100.0	100.0	100.0	100.0
Gemella hemolysans	0	0	0	23.8	28.6
Streptococcus spp.	100.0	100.0	100.0	100.0	100.0
Gram-negative facultative anaerobic cocci	12.5	0	4.8	0	0
Neisseria spp.	12.5	0	4.8	0	0
Gram-positive anaerobic cocci	62.5	71.4	52.4	61.9	76.2
Gemella morbillorum	0	23.8	28.6	38.1	38.1
Parvimonas micra	62.5	42.9	19.0	38.1	47.6
Streptococcus intermedius	25.0	23.8	19.0	9.5	19.0
Gram-positive facultative anaerobic rods	87.5	100.0	100.0	90.5	85.7
Actinomyces spp.	87.5	95.2	100.0	90.5	85.7
Lactobacilli spp.	12.5	23.8	19.0	0	0
Rothia dentocariosa	0	28.6	23.8	0	0
Gram-positive anaerobic rods	62.5	66.7	57.1	52.4	57.1
Actinomyces israelii	62.5	33.3	28.6	19.0	19.0
Bifidobacterium spp.	12.5	9.5	28.6	28.6	9.5
Eubacterium spp.	37.5	42.9	19.0	4.8	33.3
Gram-negative anaerobic cocci	87.5	57.1	76.2	61.9	38.1
Veillonella spp.	87.5	57.1	76.2	61.9	38.1
Gram-negative facultative anaerobic rods	75.0	100.0	100.0	100.0	76.2
Capnocytophaga spp.	75.0	100.0	100.0	81.0	71.4
Eikenella corrodens	37.5	28.6	19.0	33.3	23.8
Haemophilus spp.	25.0	14.3	19.0	4.8	0
Gram-negative anaerobic rods	100.0	100.0	100.0	100.0	95.2
Bacteroides spp.	12.5	9.5	4.8	14.3	19.0
Bilophilla wadworthia	25.0	4.8	4.8	0	0
Campylobacter spp.	0	23.8	19.0	23.8	23.8
Wollinella spp.	12.5	9.5	14.3	0	0
Fusobacterium spp.	75.0	90.5	85.7	42.9	52.4
Porphyromonas gingivalis	37.5	9.5	4.8	4.8	9.5
Prevotella intermedia/nigrescens	62.5	42.9	42.9	47.6	38.1
Prevotella loeschii	37.5	28.6	23.8	33.3	28.6
Prevotella melaninogenica	0	0	0	28.6	33.3
Prevotella oralis	0	0	0	19.0	28.6
Selenomonas spp.	37.5	19.0	19.0	9.5	9.5
Bacterial species IF <12.5	37.5	33.3	47.6	0	0
Staphylococcus epidermidis	0	4.8	9.5	0	0
Stomatococcus spp.	12.5	4.8	9.5	0	0
Corynobacterium matrucotii	12.5	4.8	4.8	0	0
Clostridium spp.	12.5	9.5	9.5	0	0
Leptotrichia buccalis	12.5	4.8	4.8	0	0
Porphyromonas assaccharolytica	0	4.8	4.8	0	0
Propionibacterium spp.	0	9.5	12.5	0	0

Values are given as IF%.
IF, Isolation frequency; IC, in the cleft; NC, neighbouring the cleft; MC, molars of subjects with cleft; UIC, upper incisors and canines of children in the control group; M, molars of subjects in the control group.

amount of plaque was found in children with cleft ($P < 0.005$). The differences between groups were consistent irrespective the age of the subjects. These data are in accordance with other studies in which a larger amount of dental plaque was observed in children with cleft compared with normal children[12,13,21]. Furthermore, our findings confirm the results of other studies where the PlI was found higher in sites with cleft compared with noncleft sites of the same child[4,8]. These findings can be attributed to factors that hinder proper oral hygiene such as the cleft deformity, collapse of the maxillary segments,

Table 9. Relative proportion (mean %) of bacterial species in test and control groups in the subgingival plaque of teeth in the cleft, teeth neighbouring the cleft, molars of children with cleft, the upper incisors and canines of children in the control group, and molars of children in the control group.

	Test group			Control group	
Bacterial species	**IC (*n* = 8)**	**NC (*n* = 21)**	**MC (*n* = 21)**	**UIC (*n* = 21)**	**M (*n* = 21)**
Gram-positive facultative anaerobic cocci	28.9* ± 8.9	32.4* ± 9.9	32.8** ± 8.9	39.3* ± 8.7	38.9** ± 9.5
Gemella hemolysans	0 ± 0	0 ± 0.0	0 ± 0	2.5 ± 4.8	2.8 ± 5.2
Streptococcus spp.	28.9 ± 28.9	32.4 ± 9.9	32.8 ± 8.9	36.8 ± 9.3	36.1 ± 10.9
Gram-negative facultative anaerobic cocci	0.9 ± 0.9	0 ± 0.0	0.2 ± 1.1	0 ± 0	0 ± 0
Neisseria spp.	0.9 ± 0.9	0 ± 0	0.2 ± 1.1	0 ± 0	0 ± 0
Gram-positive anaerobic cocci	4.5* ± 5.4	6.5 ± 5.3	4.3** ± 4.6	8.2* ± 9.0	10.0** ± 10.4
Gemella morbillorum	0 ± 0	2.1 ± 4.2	2.3 ± 4.0	5.4 ± 7.8	6.5 ± 8.8
Parvimonas micra	3.0 ± 2.9	2.2 ± 3.0	0.8 ± 1.7	1.7 ± 2.5	1.9 ± 2.6
Streptococcus intermedius	1.5 ± 3.3	2.2 ± 4.1	1.2 ± 2.5	1.1 ± 3.5	1.6 ± 3.5
Gram-positive facultative anaerobic rods	15.6 ± 7.7	17.6 ± 6.6	17.8** ± 5.0	14.1 ± 7.5	13.6** ± 8.8
Actinomyces spp.	14.3 ± 6.7	15.1 ± 7.4	15.9 ± 3.0	14.1 ± 7.5	13.6 ± 8.8
Lactobacilli spp.	1.3 ± 3.6	0.9 ± 1.7	0.7 ± 1.4	0 ± 0	0 ± 0
Rothia dentocariosa	0 ± 0	1.6 ± 3.6	1.2 ± 2.5	0 ± 0	0 ± 0
Gram-positive anaerobic rods	4.6 ± 4.8	3.8 ± 3.6	3.1 ± 3.2	3.3 ± 3.5	3.5 ± 3.7
Actinomyces israelii	2.4 ± 2.0	1.3 ± 2.1	0.9 ± 1.6	1.1 ± 2.3	1.0 ± 2.2
Bifidobacterium spp.	0.8 ± 2.2	0.5 ± 1.5	1.2 ± 2.1	2.0 ± 3.4	0.4 ± 1.2
Eubacterium spp.	1.4 ± 2.4	2.0 ± 2.8	1.0 ± 2.2	0.3 ± 1.3	2.1 ± 3.3
Gram-negative anaerobic cocci	5.8 ± 3.0	4.9 ± 4.9	5.6 ± 3.9	6.3 ± 5.3	4.2 ± 5.6
Veillonella spp.	5.8 ± 3.0	4.9 ± 4.9	5.6 ± 3.9	6.3 ± 5.3	4.2 ± 5.6
Gram-negative facultative anaerobic rods	11.4 ± 8.4	13.8 ± 4.1	15.2** ± 6.4	12.2 ± 6.9	10.8** ± 8.1
Capnocytophaga spp.	8.1 ± 5.8	11.3 ± 2.2	12.1 ± 2.5	10.0 ± 6.0	9.4 ± 7.0
Eikenella corrodens	2.1 ± 3.0	1.6 ± 3.1	1.4 ± 3.2	1.8 ± 2.9	1.4 ± 2.7
Haemophilus spp.	1.2 ± 2.6	0.9 ± 2.4	1.7 ± 4.1	0.4 ± 1.7	0 ± 0
Gram-negative anaerobic rods	26.2* ± 16.2	18.5 ± 6.4	17.3 ± 8.2	16.6* ± 8.5	18.8 ± 11.2
Bacteroides spp.	0.7 ± 2.0	0.5 ± 2.0	0.2 ± 0.8	0.7 ± 1.8	1.0 ± 2.5
Bilophilla wadworthia	1.1 ± 2.0	0.2 ± 1.3	0.2 ± 0.7	0 ± 0	0 ± 0
Campylobacter spp.	0 ± 0	1.1 ± 2.4	1.1 ± 2.4	1.8 ± 3.5	1.8 ± 3.3
Wollinella spp.	0.7 ± 2.1	0.6 ± 1.8	0.7 ± 1.8	0 ± 0	0 ± 0
Fusobacterium spp.	9.1 ± 6.0	9.7 ± 4.3	10.3 ± 5.1	3.7 ± 4.8	4.7 ± 5.0
Porphyromonas gingivalis	3.2 ± 5.3	0.7 ± 2.6	0.2 ± 1.1	0.5 ± 2.4	0.8 ± 2.4
Prevotella intermedia/nigrescens	5.7 ± 3.1	3.2 ± 4.2	2.7 ± 4.0	3.5 ± 3.8	2.9 ± 3.9
Prevotella loeschii	3.8 ± 5.4	1.9 ± 3.3	1.3 ± 2.4	3.1 ± 4.6	3.0 ± 5.0
Prevotella melaninogenica	0 ± 0	0 ± 0	0 ± 0	2.1 ± 3.5	2.9 ± 4.4
Prevotella oralis	0 ± 0	0 ± 0	0 ± 0	1.0 ± 2.2	1.5 ± 2.5
Selenomonas spp.	1.9 ± 2.9	0.7 ± 1.8	0.6 ± 1.4	0.2 ± 0.8	0.2 ± 0.8
Bacterial species IF <12.5	1.9 ± 3.3	2.3 ± 3.7	3.7 ± 4.6	0 ± 0	0 ± 0
Staphylococcus epidermidis	0 ± 0	0.2 ± 1.1	0.5 ± 1.7	0 ± 0	0. ± 0
Stomatococcus spp.	0.7 ± 2.0	0.3 ± 1.5	0.5 ± 1.9	0 ± 0	0 ± 0
Corynobacterium matrucotii	0.4 ± 1.2	0.2 ± 0.7	0.5 ± 2.1	0 ± 0	0 ± 0
Clostridium spp.	0.5 ± 1.5	0.7 ± 2.7	0.7 ± 2.9	0 ± 0	0 ± 0
Leptotrichia buccalis	0.3 ± 0.8	0.1 ± 0.3	0.1 ± 0.2	0 ± 0	0 ± 0
Porphyromonas assaccharolytica	0 ± 0	0.4 ± 1.7	0.6 ± 2.7	0 ± 0	0 ± 0
Propionibacterium spp.	0 ± 0	0.4 ± 1.3	0.9 ± 1.9	0 ± 0	0 ± 0
Gram-positive total	55.3* ± 9.7	61.8 ± 7.1	60.1** ± 9.1	64.9* ± 9.3	66.1** ± 12.5
Gram-negative total	44.7* ± 9.8	38.1 ± 6.9	39.9** ± 8.3	35.1* ± 9.2	33.9** ± 12.5

Values are given as mean% ± SD.

*Statistically significant difference in upper incisors and canines $P < 0.05$.

**Statistically significant difference in molars $P < 0.05$.

IC, in the cleft; NC, neighbouring the cleft; MC, molars of subjects with cleft; UIC, upper incisors and canines of children in the control group; M, molars of subjects in the control group.

orthodontic anomalies, scarring, and inelastic upper lip as a result of corrective surgeries. Only Lucas and co-workers[10] reported no significant differences in the oral hygiene level of children with cleft and normal children of the control group, which is probably attributed to the fact that all children in their study participated in a preventive dental

programme and the PlI was assessed by only visual examination.

In contrast with the differences in the PlI between the two groups of subjects there were no statistically significant differences in the mean GI ($P > 0.05$), as all study subjects presented a mild degree of gingival inflammation. The subjects with cleft did, however, show more of an increase in inflammation during adolescence. These results concur with those of Lucas and co-workers,[10] but disagree with other authors who reported a significantly higher degree of gingivitis in children with cleft both in the upper anterior region, approximating the cleft as well as when the posterior areas were compared[4,7,8]. A possible explanation for these contradictory findings is the fact that most children and adolescents, in both groups, of our study (75.6%) were undergoing orthodontic treatment which in conjunction with the moderate oral hygiene level can result in gingival inflammation. Furthermore, none of the subjects in our study participated in an individualised preventive dental programme (i.e., use of antimicrobials or intensified use of topical fluorides), other than the standard preventive programme.

The CPITN index was recorded for the first time in children and adolescents with cleft in our study. The majority of study subjects in both groups (68% test group and 78% control group) presented calculus, which can be attributed to the fair to moderate oral hygiene level. More children and adolescents with cleft (31%) had PPD scores up to 5.5 mm in at least one sextant in their mouth compared to only (5%) of control subjects, probably because of the increased amount of plaque in children with cleft and the long-term orthodontic treatment.

According to the results of our study the mean PPD score of the upper anterior teeth neighbouring the cleft (NC) and teeth in the cleft (IC) was up to 3 mm, which is considered within normal limits and is in accordance with the results of Dewinter and co-workers,[11] whereas Quirynen and co-workers[2] found only slightly increased PPD of teeth in the cleft region (± 0.50 mm higher than the contra-lateral control teeth). When the PPD of upper anterior teeth in the two groups of children was compared, there was a significantly increased PPD of teeth in the cleft region ($P < 0.006$). This is in accordance with Al-Wahadni and co-workers[21] who reported PPD of teeth in the cleft region up to 3.7 mm, in children and adolescents 10–15 years old. The results of this study are similar to the findings of other authors who conducted similar studies in adult subjects with cleft and found that the periodontal condition of upper front teeth in the cleft region is deteriorated compared with the other teeth as shown by the increased PPD and radiographic loss of bone height[4,5].

In our study a higher percentage of surfaces of teeth in the cleft area presented bleeding on probing compared with the upper anterior teeth of control children. Those findings are in accordance with other authors and are probably because of the increased amount of plaque in the cleft region[4,5,19].

The increased mobility of teeth in the cleft area compared with the corresponding teeth of the control group was attributed to the reduced amount of supporting bone as shown by another study[22]. This speculation, however, can be confirmed only by radiographic examination, which was not performed in our study.

The composition of bacterial dental plaque and the presence of specific putative periodontal pathogens have been related to increased risk for progressive periodontal disease. As the cleft region harbours retentive sites for dental plaque accumulation, because of the anatomy of the region and the resulting difficulty in oral hygiene practice, the question arises whether children with cleft could be colonised earlier by putative periodontal pathogens. One of the objectives of this study was to describe the subgingival microbial profile of children and adolescents with cleft and to determine whether they are colonised by microorganisms implicated in periodontal disease to a greater extent than their counterparts of the control group.

According to the results of the microscopic examination, teeth in the cleft harboured higher proportions of motile bacteria and spirochetes compared with teeth neighbouring the cleft ($P < 0.05$) and the corresponding

teeth of the control group ($P > 0.05$). Those findings are in accordance with the results of other authors, who found that teeth in the cleft region presented higher proportions of motile bacterial forms compared with the corresponding contra-lateral opponents[2,6]. Furthermore, the high percentage of subjects undergoing orthodontic therapy in our study is probably related to the above results as it has been shown that the subgingival microbiota of teeth with orthodontic brackets harbours higher percentages of motile bacteria and spirochetes[23].

The mean total anaerobic count of the major cultured bacterial groups, was higher in the control group of our study, both in the upper anterior teeth ($P > 0.05$) and the molars ($P < 0.05$), which can probably be related to factors such as the frequency and the technique of tooth brushing or the PPD of the molars, but the above factors were not examined separately in our study.

The microbiological cultures detected high relative proportions of *Streptococcus* and *Actinomyces* spp. in all sites of both groups of children and adolescents, which is in accordance with other authors, as those species are among the early colonisers of tooth surfaces and important components of dental plaque associated with the maintenance of periodontal health[24].

Capnocytophaga spp. were very frequently isolated in our study in the subgingival microbiota of all sites [Isolation Frequency (IF) 81–100%] in high relative proportions (10–12.1%). They seem to be major colonisers of children's and adolescents' gingival sulci as shown by similar studies in healthy subjects[25–27].

The Gram-negative anerobic bacteria, some of which are considered putative periodontal pathogens, were found in high isolation frequencies (100%) and high relative proportions (26.2%) in teeth in the cleft compared with the corresponding teeth of the control group. *Fusobacterium* spp. was commonly found in children and adolescents with cleft (IF: 75–90.5%, RP: 9.1–10.3%), in higher proportions compared with their noncleft counterparts. This species has been found to be the most common recovered species in the gingival crevice. *Fusobacterium nucleatum* has been isolated from periodontally healthy as well as diseased sites, demonstrating both phenotypic and genotypic diversity within the species and possible differences in pathogenic potential among subspecies[28]. Frisken and co-workers[29] isolated this species from 60% to 70% of children 5–7 years of age. Its presence in the subgingival microbiota is important as it possesses receptors for its binding with both nonpathogenic as well as pathogenic species and is a perquisite for the organisation of a more complex oral biofilm. *Prevotella intermedia/nigrescens* was more frequently isolated in the subgingival microbiota of children with cleft (IF: 62.5%) compared with the control group (47.6%). Moore and co-workers[25] observed that *Bacteroides intermedius*, currently *P. nigrescens*, was more common in children than in adults, but later they listed it as possible pathogen connected with periodontitis. In this study it could not be differentiated whether it was *P. intermedia* or *P. nigrescens* because of the culture technique used.

Porphyromonas gingivalis, which is considered a periodontal pathogen was isolated in higher IF (32.5%) but in low relative proportion (3.2%) in teeth in the cleft, compared with other sites. This is in agreement with Gafan and co-workers[30] who isolated *P. gingivalis* in the subgingival microbiota of periodontally healthy children up to 11 years old (IF 14–50%). By contrast, this disagrees with the results of Costa and co-workers[13] who did not detect *P. gingivalis* in any of the children with cleft. Divergence among the studies may be because of technical variability, differences in sample collection, or the probability of different colonisation models throughout the world.

The absence of *A. actinomycetemcomitans* in the children and adolescents of this study is in agreement with other studies in subjects with cleft[2,6]. In conclusion, the present results indicate that children and adolescents with cleft harbour a multiform oral Gram-negative anaerobic bacteria some of which are suspected periodontal pathogens. Their isolation frequencies and relative proportions are increased in the subgingival microbiota of

teeth in the cleft. It can be speculated that children with cleft, colonised early with periodontal pathogenic species, may be at greater risk for the outbreak of periodontal disease, either in childhood or later in life. The microbiological and clinical results of this study emphasise the importance of a regular recall programme that should start early in the life of children with cleft, and should be integrated in their treatment protocol.

What this paper adds

- Although the sub-gingival microbiota of children with cleft lip/palate does not show major differences, when compared with normal matched children, a tendency for increased isolation frequencies and relative proportions of putative pathogens is apparent in young children with clefts.

Why this paper is important to paediatric dentists

- Children with cleft lip/palate should be placed in an intensive preventive programme from an early age to avert the establishment of a pathogenic microbiota that could lead to periodontal disease later in life.

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