RESEARCH ARTICLE

Some oral microbiota in complete cleft infants in comparison with normal infants

Rula Qays Abdulkareem Al-Humairi, Zainab Juma Jafar

Abstract

Objective: To compare caries-related microorganisms and candida in complete cleft infants with normal palate infants.

Method: The case-control study was conducted from April 2021 to January 2022 at the College of Dentistry, University of Baghdad, Baghdad, Iraq, and comprised infants with age ranging from 1 day to 4 months. They were divided into complete cleft group A and control group B. Group A was subdivided into those with class III Veau's palatal classification, and the rest with class IV Veau's palatal classification. Samples were taken using oral swab which were subjected to colony morphology, gram staining and biochemical testing. Data was analysed using SPSS 21.

Result: Of the 52 subjects, 26(50%) were in each of the 2 groups. The subgroups of group A had 13(50%) patients each. The counting and colonisation of streptococcus mutans, lactobacilli and candida albicans were significantly higher in group A than in group B ($p<0.05$). The difference between the subgroups of group A was not significant ($p>0.05$).

Conclusion: Infants with cleft palate were more susceptible to dental caries and oral infection than those with normal palate.

Key Words: Candida albicans, Streptococcus, Cleft Palate, Dental, Lactobacillus, Staining

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Introduction

During the early hours post-birth, the mouth, which stays sterile during foetal development, transforms into a varied environment occupied by various bacteria. Microbiota invades the skin and mucous membranes of the newborns as a consequence of interaction with the external environment.[1] In the early neonatal period, a large portion of the oral microbiota comes from the mother, and is a transitory community of microorganisms composed mostly of gut bacteria in normally born neonates.[2] The resident microbiota during this time period is mostly determined by external variables, such as gestational age, birth style, type of feeding, duration of hospital stay after delivery, and overall health.[3] The multifarious assembly of the oral cavity, with multiple folds, mucosal invaginations of the palate, cheeks and tongue creating niches along with varying oxygen concentrations and potential of hydrogen (pH) values, buffer capacities, ionic compositions, redox state hydration and salivary access. These circumstances foster the formation of a diversified ecosystem based on bacterias' interactions with their host environment.[4]

Department of Paediatric and Preventive Dentistry, University of Baghdad, Baghdad. Iraq.

Correspondence: Rula Qays Abdulkareem Al-Humairi

Email: roula.qays93@gmail.com

Within a few hours of birth, the oral microbiota is colonised by viridans streptococci and streptococcus (S.) salivarius, which are commensals that colonise the mouth cavity permanently.[5] Congenital orofacial malformations impact the oral cavity's structure and function, altering its features dramatically. As a consequence, such deformities may have an effect on the environment's microbiota.[6] Orofacial clefts are the most frequent congenital oral cavity developmental abnormality.[7] Complete cleft palates in neonates are defined by the involvement of the main and secondary palates entirely. It stretches all the way from the uvula to the alveolar ridge. It is a condition that affects both the main and secondary palates. Cleft palates might be unilateral or bilateral.[8] Additionally, newborns and babies with orofacial cleft need specialised care to ensure adequate cleanliness of the oro-nasal passages, with an emphasis on preparation for future surgical treatments.[9] Changes in the ecosystem have been reported in different classifications of clefts in primary or permanent dentition, in addition to the involvement of different types of surgical or orthodontic treatment.[10] Both the aberrant anatomy and dysfunction of the oral cavity in babies with cleft palate generate an environment that is unlike that of healthy neonates. As a result, these anomalies may have an effect on the oral microbiome.[11]

Dental caries is a chronic bacterial infection of the hard tissues of teeth implicating several types of oral bacteria.[12] The most important microorganisms involved in dental caries are S. mutans, lactobacillus and actinomycetes.[13] Lactobacillus have little or no effect on caries initiation, but play an essential role in the progression of caries.[14]

The current study was planned to compare caries-related microorganisms and candida in complete cleft infants with normal palate infants.

Patients and Methods

The case-control study was conducted from April 2021 to January 2022 at the College of Dentistry, University of Baghdad, Baghdad, Iraq, and was approved by the ethics committee ERC No. 316 on 24/3/2021.The study comprised infants with age ranging from 1 day to 4 months who were enrolled from the maternity and children's hospitals and from the departments of Plastic Surgery in some hospitals in Baghdad city. The controls were enrolled from primary healthcare centres. The study sample was raised using a simple random sampling technique. The subjects were divided into complete cleft group A and control group B. Group A was subdivided into those with class III Veau's palatal classification, and the rest with class IV Veau's palatal classification.[15]

The sample size was calculated using G*power version 3.1.9.7.[16] with a power 95%, alpha error of probability of 0.05, assumed Cohen's r effect size of 0.5, and adding 10% error rate [17]

After obtaining consent of the parents, baseline data was collected using a questionnaire about the birth date, if the infant was on any medication or had any systemic disease. Those included were infants with complete cleft palate before surgery as well as healthy infants with normal palate. Those excluded were patients with the coexistence of orofacial cleft (except the cleft lip) with other developmental abnormalities, infants with any systemic disease, and those on antibiotic therapy for the preceding 2 weeks.

Oral examination was done, and the type of cleft was recorded according to Veau's classification for palatal cleft. Class III Veau palatal classification is characterised by the involvement of both soft and hard palates and the alveolar process on one side of the premaxillary area, while class IV involves both soft and hard palates and continues through the alveolus on both sides of the premaxilla, leaving it free and often mobile.

Oral samples were taken by oral swab from the palatal mucosa near the cleft area for each infant in group A, and from the palatal side for each infant in group B. The samples were transferred immediately to a tube of sterile Cary Blair transport media. The samples were then transported to the laboratory to be incubated in a medium that was prepared before taking the sample for counting and identification studies. The samples were put in phosphate-buffered saline (PBS) and homogenised for 1 minute using a vortex mixer and were subjected to 10-fold serial dilutions. Sterile microbiological spreader was used on the plates of Sp20 agar, which is a selective culture media for S. mutans, and Rogosa agar, a selective media for lactobacilli. The plates were incubated anaerobically by using a gas pack supplied in an anaerobic jar at 37◦C for 48hrs, followed by aerobic incubation for 24hrs at 37◦C in duplicate, and incubated aerobically at 37◦C for 24 hrs. Sabouraud dextrose agar was also used for the isolation of candida (C.) albicans, and was incubated aerobically at 37ºC for 48hrs. Bacterial counting was done by counting the number of colonies on a pour palate, called direct counting. The identification of microorganisms was done using colony morphology, gram staining and biochemical testing for which

A/Catalase test (slide method), B/Mannitol fermentation test, and C/Germ Tube Test of C. albicans were employed.

Data was analysed using SPSS 21. Data was expressed as mean and standard error (SE) along with minimum-maximum range, as well as median and mean rank. Shapiro-Wilk test was used for checking data normality. Spearman's correlation was used to test the monotonic relationship between two non-normally distributed variables. P<0.05 was taken as the level of significance.

Results

Of the 52 subjects, 26(50%) were in each of the 2 groups. The subgroups of group A had 13(50%) patients each. The counting and colonisation of S. mutans, lactobacilli and C. albicans were significantly higher in group A than in group B (Table). The difference between the subgroups of group A was not significant (Figure).

Table: Intergroup comparisons related to streptococcus (S.) mutans, lactobacilli and candida (C.) albican.

Groups			Wilcoxon Sum rank test	P value
S. mutans				
Cleft group			3.517	0.00044*
	Minimum	0		
	Minimum	17		
	Median	7.00		
	Mean rank	33.85		

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Normal group				
	Minimum	0		
	Maximum	11		
	Median	5.00		
	Mean rank	19.15		
Lactobacilli			2.422	0.015*
Cleft group				
	Minimum	0		
	Maximum	34		
	Median	20.50		
	Mean rank	31.58		
Normal group				
	Minimum	0		
	Maximum	29		
	Median	11.50		
	Mean rank	21.42		
Candida albican			2.153	0.013*
Cleft group				
	Minimum	7		
	Maximum	47		
	Median	28.5		
	Mean rank	31.02		
Normal group				
	Minimum	5		
	Maximum	43		
	Median	18		
	Mean rank	21.98		

*=significant at $p<0.05$

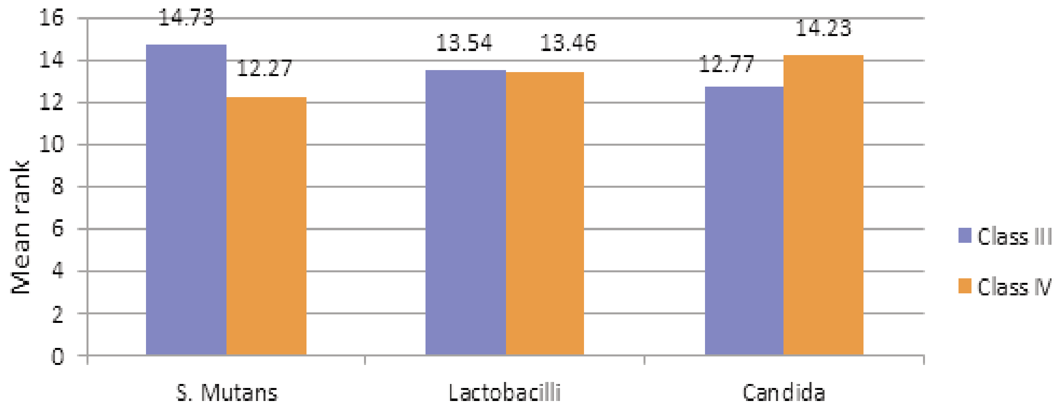

Figure: Microflora counts between Veau's palatal classification classes within the cleft palate group..

Discussion

The infants in the current study had no systemic disease and were not taking any medication, which was done to exclude the eradication of some microorganisms or allowing the over-growth of others, which may eventually cause shifting in the oral microflora.[18] S. mutans count was higher in cleft group than in the normal palate group. The count of S. mutans and lacbacilli was higher in class III than in class IV, while other flora's count was higher in class IV compared to class III, but the difference was not significant. The finding agreed with that of a previous study.[19]

S. mutans are considered to be the major pathogens in the initiation of dental caries, divide the sucrose in food, and utilise one of the sugars to build a capsule that adheres tightly to the tooth.[20] The bacterias that are trapped in the capsule use the remaining sugar to fuel their metabolism and generate lactic acid that attacks the tooth enamel.[21] A study reported that the cleft lip and/or palate patients had major oral health problems, such as dental caries in both primary and permanent dentition, and needed treatment.[22]

In the current study, lacobacilli count was higher in cleft group than in the normal palate group, and lactobacilli count was higher in class III than in class IV cleft. Traditionally, lactobacilli are the most important microorganisms concerned in progression of dental caries.[23] There is an established strong correlation between lactobacillus count in saliva and higher Decayed-Missing-Filled (DMF) index in children with high lactobacillus count.[24] Children with cleft are suspected to be more susceptible to dental caries, higher DMF index, and more treatment needs, which is in agreement with previous studies[25,26].

The current study revealed that C. albican count in infants with cleft palate was significantly higher than in healthy controls. The colonisation rate of candida and the distribution of C. albicans were not significantly related to the classes of cleft group. This agrees with earlier findings.[27] A study showed that infants with cleft palate had a significantly worse health condition and were more susceptible to oral infection than healthy controls.[28]

Limitation: The current study has limitations as the sample was taken using oral swabs instead of the recommended saliva drooling, which was owing to the difficulty of communicating with the infants.

Conclusion

Infants with cleft palate were found to be more susceptible to dental caries and oral infection than the healthy controls due to the high prevalence of caries-related microorganisms and candida.

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Conflict of Interest: None.

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