



Selected Salivary Physicochemical Properties among Cleft Lip and Palate Children in Iraq

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ABSTRACT

Objective: To investigate selected salivary properties in children with cleft lip and palate and to compare them with non-cleft children. **Setting:** The study was conducted in Dentistry College, University of Baghdad and Alwasity and Ghazi Alhareery Teaching Hospitals, Baghdad, Iraq. **Patients and methods:** A total of 36 children with non-syndromic repaired cleft lip and palate, aged 4 to 10 years, and a total of 37 non-cleft children were investigated for flow rate, pH, total antioxidant, uric acid, and total proteins. **Results:** Salivary total antioxidant capacity and total protein were significantly different between cleft lip and palate children and non-cleft children, while for salivary flow rate and total antioxidant were significantly lower in children with cleft lip and palate than in non-cleft children. Concerning pH and uric acid, there were no significant differences. **Conclusion:** Some salivary chemical constituents and some physical parameters deviated from the norm in children with cleft lip and palate.

Keywords: Cleft lip and palate, Salivary flow rate, pH, Total antioxidant, Uric acid, Total proteins

INTRODUCTION

Orofacial cleft (OFC) is a congenital abnormal space or gap due to incomplete fusion of the embryologic prominences in the upper lip alveolus and/or palate during facial development which results in severe facial, morphological and functional impairment of sucking, swallowing and breathing. OFC is the most common craniofacial birth defects in humans [1,2]. The craniofacial structures development is a coordinated process involving the growth of multiple independently derived embryologic prominences called primordial. Incomplete fusion of these facial structures during the 4th to 12th week of embryologic life results in a gap which leads to cleft lip, cleft of the primary or secondary palate, or a combination of them [3,4]. Elevated infant mortality and significant lifelong morbidity are associated with OFC such as cosmetic deformities, feeding problems, swallowing difficulties, failure to gain weight, change in nose shape, recurrent ear infections, poor growth of the maxilla, speech difficulties, misaligned teeth and dental abnormalities [2,5,6].

Saliva has many essential functions such as lubrication, protection, buffering action, clearance and antibacterial activity so that saliva plays an important role in regulating and maintaining the integrity of the oral hard and soft tissues and in oral health monitoring [7]. The whole saliva offers an alternative to serum as a biologic fluid that can be analyzed for diagnostic purposes, so saliva has been investigated as a source of specific biomarkers for human disease such as cancer, periodontitis and in cleft lip palate children [8,9]. Saliva collection is a simple, inexpensive and non-invasive method [2].

Antioxidants are present in saliva, which constitutes the first line of defense against oxidative stress and has protective effects against microorganisms, toxins, and oxidants. The imbalances in free radical levels and reactive oxygen species with antioxidants may play a key role in the onset and development of several inflammatory oral pathologies [10]. Saliva has an antioxidant system that includes macromolecules, enzymes and an array of small molecules [11]. Uric acid is the most important antioxidant molecule, contributing approximately 70% of the total salivary antioxidant capacity. The antioxidant properties of uric acid have been attributed to its ability to chelate transition metals and to react with biological oxidants [8].

The protein and peptides composition of saliva play an important role in preserving the integrity and stability of the saliva and providing defense against infections and diseases such as periodontal disease or head and neck squamous

carcinoma [2,12]. Numerous physiological and pathological factors can cause an alteration in the salivary flow rate. Clinically significant oral discomfort results when salivary flow rate decreased [7]. Buffering capacity of saliva plays an important role in the organization of oral microbiota because they maintain the salivary pH at normal range. Diminished pH encourages salivary protein acid precipitation, which offers an organic base for plaque accumulation and subsequent dental caries [13-15].

PATIENTS AND METHODS

Subjects

This study was carried out in Baghdad city, Iraq. Informed consent was obtained from each participant enrolled in this study before any data collection and examination of oral health. Two groups were examined with age range (4-10) years. The study group included 36 children, which matched the inclusion criteria and attended Alwasity and Ghazi Alhareery Teaching Hospitals, Maxillofacial departments. The inclusion criteria of the study group include non-syndromic OFC; surgically repaired; cleft with bone involvement; free from any systemic disease. They were classified according to Elmassry classification [16]. The control group included 37 non-cleft children free from any systemic disease who attended Pedodontics department at Baghdad Dentistry College. Control group was matched with a study group in age and gender.

Saliva Analysis

For each child, saliva collection was done in the morning (9-12 AM). Unstimulated whole saliva was collected in sterile graduated test tubes under standardized conditions following the instruction reported by Navazesh, et al. [17]. The salivary flow rate was determined after foam fading by dividing the total volume of collected saliva in milliliter on the total time of saliva collection in a minute [18]. Digital pH meter with automatic temperature compensation was used to measure the pH of the unstimulated whole saliva. After collection and determination of flow rate and pH, the saliva was stored at -20°C till the analysis day. The frozen salivary samples were allowed to thaw and come to room temperature and centrifuged at 3000 rpm for 10 min. The biochemical analysis of salivary samples was carried out at Medical Laboratory. Total antioxidant kit manufactured by Cayman Chemical was used for measurement of total antioxidant in saliva using Enzyme Linked Immune Sorbent Assay (ELISA) machine. Total protein kit manufactured by SPINREACT was used for measurement of total protein in saliva by the enzymatic colorimetric manual method. Uric acid kit manufactured by RANDOX was used for measurement of uric acid in saliva.

Statistical Analysis

All data were analyzed and presented using statistical package for social science software (SPSS version 21, USA). Descriptive statistics were used to summarize frequencies and percentages of nominal variables, while mean and standard deviation (SD) for quantitative variables. The difference in salivary parameters was tested using independent sample test. The level of significance for all tests was set as non-significant at $p > 0.05$, significant at $p \leq 0.05$, and highly significant at $p < 0.01$.

RESULTS

In the present study, two groups were examined: the study group, which consisted of 36 children with OFC and the control group, which consisted of 37 non-cleft children (Table 1).

Table 1 Distribution of the studied sample

Cleft and normal control				
Subjects	Cleft (n=36)		Non-cleft (n=37)	
Gender				
Boys	18 (50.0%)		18 (48.6%)	
Girls	18 (50.0%)		19 (51.0%)	
Age				
≤ 7.5	19 (52.8%)		17 (45.9%)	
>7.5	17 (47.2%)		20 (54.1%)	
Cleft children according to cleft type				
Subjects	UCLA ^a	BCLA ^b	UCLP ^c	BCLP ^d

Gender				
Boys	0 (0.00%)	0 (0.00%)	5 (13.89%)	13 (36.11%)
Girls	3 (8.33%)	1 (2.78%)	6 (16.67%)	8 (22.22%)
Age				
≤ 7.5	3 (8.33%)	1 (2.78%)	4 (11.11%)	11 (30.56%)
> 7.5	0 (0.00%)	0 (0.00%)	7 (19.44%)	10 (27.78%)

UCLA^a=Unilateral cleft lip and alveolus; BCLA^b=Bilateral cleft lip and alveolus; UCLP^c=Unilateral cleft lip and palate; BCLP^d=Unilateral cleft lip and palate

The distribution of the study group according to OFC classification is shown in Table 1. This table shows that BCLP was the most common (58.33%), followed by UCLP (30.56%) and UCLA (8.33%) whereas the less common was BCLA (2.78%).

Salivary flow rate (ml/min) and pH among study and control groups (mean ± SD) is illustrated in Table 2. This table shows that the mean value of salivary flow rate was found in the study group (0.16) which is lower than the control group (0.27) with the statistically highly significant difference between two groups ($p < 0.01$). No significant difference concerning salivary pH was found between the study and the control groups ($p > 0.05$).

Table 2 Salivary flow rate (ml/min), pH and salivary concentrations of uric acid (ml/dl), total protein (ml/dl) and total antioxidant capacity (mM) among study and control groups

Saliva parameters	Mean ± SD		
	Cleft (n= 36)	Non-cleft (n= 37)	p-value
Flow rate	0.16 ± 0.15	0.27 ± 0.18	0.006**
pH	7.83 ± 0.61	7.74 ± 0.88	0.593
Uric acid	1.87 ± 0.86	1.64 ± 0.75	0.223
Total protein	23.40 ± 3.79	20.51 ± 3.82	0.002**
Total antioxidant capacity	0.08 ± 0.09	0.31 ± 0.17	0.000**

**Highly significant at $p < 0.01$

Table 2 also illustrates the mean values and standard deviations of the salivary concentration of uric acid (ml/dl), total protein (ml/dl) and total antioxidant capacity (mM) between study and control groups. The table shows that no significant difference concerning salivary UA was found between study and control groups ($p < 0.01$). The same table shows that the mean values of salivary total protein were found to be higher in study group (23.4) than control group (20.51) with highly significant difference between study and control groups ($p < 0.01$), while the mean values of total antioxidant capacity in the same table was lower in the study group (0.08) than control group (0.31) with highly significant difference between study and control groups ($p < 0.01$).

DISCUSSION

The current study showed a highly significant decrease in mean value of salivary flow rate between study group and control group; this agreed with Dahllof, et al., and disagreed with Cheng, et al. [19-22]. This difference is difficult to explain but may be attributable to difficulties in spitting caused by the scar tissue in the upper lip, by a defective orbicularis oris muscle or by problems in achieving proper closure of the nasopharynx, also constriction of appliances for occlusal disturbances correction decrease saliva flow [14,19]. Concerning the pH of saliva, this study showed that the average pH of both groups was over 7, thus indicating no significant difference between the OFC group and control group. This finding agreed with Cheng, et al., and controversy with and Deshpande, et al. [9,20].

Total antioxidant capacity (TAC) measurement has been widely investigated for assessment of oxidative status in persons with different diseases. Any deficiency in one of the components of the antioxidant system can reduce the overall individual's antioxidant capacity [23]. In the present study, the mean value of TAC was lower in the study group than the control group with a statistically highly significant difference and this agreed with the findings of Aizenbud, et al. [21]. Preethi, et al., suggested that the levels of antioxidants could be altered in response to an infection or disease [24]. Assumptions by Aizenbud, et al., show that genetical alteration of some salivary antioxidant transport occurs in OFC children or oronasal fistula that resulted from early closure of the palatal cleft which may create constant communication between oral and nasal cavity, and can cause unique oral biology environment differed from normal persons [21].

Regarding salivary total protein, the mean value was higher in the study group than the control group with a statistically highly significant difference and this agreed with the findings of Aizenbud, et al., and Deshpande, et al., [9,21]. Several common secreted proteins such as actin, salivary cystatins, keratins, and other keratinocyte-activating proteins were upregulated by a cleft, which is of vital importance in the general protection and tissue regeneration of patients with cleft lip and palate [2,9].

There was no difference in the mean values of uric acid concentration in the study and the control groups, these findings disagreed with Aizenbud, et al., [21]. Uric acid may be increased due to a respective decrease in total antioxidant capacity of the oral cavity to compensate for increased oxidative stress [21].

CONCLUSION

Salivary analysis revealed that total antioxidant capacity and total protein were significantly different in orofacial cleft children in comparison to non-cleft children, but there was no difference in uric acid concentration between them. Concerning flow rate and the pH reflected, the flow rate was greatly decreased in orofacial cleft children, while pH was within the normal limit for both healthy and orofacial cleft children.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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