International Journal of Paediatric Dentistry 2006; **16:** 186–191

Total antioxidant levels of saliva in children related to caries, age, and gender

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Summary. *Aim.* The purpose of this study was to evaluate the relationship between the physicochemical properties of saliva such as flow rate, buffering capacity, pH, calcium level, total protein, total antioxidant status, and dental caries, age, and gender.

Material and methods. The antioxidant activity of saliva was investigated in 80 healthy children aged 7–15 years. They were divided into subgroups according to gender, age (7–10 years and 11–15 years), and caries activity (caries active = CA and caries free = CF). Unstimulated saliva samples were collected from all groups. Flow rates were determined, and samples analysed for pH, buffer capacity, calcium, total proteins, and total antioxidant status. Salivary total antioxidant activity (TAA) was estimated by an adaptation of the ABTS (2,2-azino-di (3-ethylbenzthiazoline-6-sulphonate)) assay.

Results. The results indicated that in general, although there was no linear association between salivary flow rate pH and buffering capacity values obtained from different groups, salivary calcium concentration values were found to be higher in caries free groups. Total protein and total antioxidant values were higher in caries active groups except those in the 11- to 15-year-old girls group.

Conclusion. In general, total protein and total antioxidant insaliva were increased with caries activity. Calcium concentrations in saliva were higher in caries-free children. In addition, calcium concentration increased with age. Thus, it can be concluded that a linear association exists between calcium concentration age and caries activity. More clinical and laboratory studies are needed to determine the exact relationship between the physicochemical properties of saliva such as flow rate, buffering capacity, pH, calcium level, total protein, total antioxidant status, and dental caries, age, and gender.

Introduction

Antioxidants are found in all biological species and protect against the potentially harmful effects of processes or reactions that cause excessive oxidations [1,2]. Therefore, biological antioxidants form an important part of our diet and together with intracellular antioxidants and antioxidant enzyme systems may prevent various pathological diseases [1,2].

The antioxidant defence systems are also highly complex. It is essential to evaluate the amounts and/or the activities of the different antioxidants when assessing antioxidant status *in vivo* [2]. One of the most important functions of saliva peroxidase is the control of oral bacteria that form dental plaque, to imbalance in the ecology and which lead and to dental caries and chronic inflammatory periodontal diseases. Many studies on saliva report the physicochemical properties of saliva (flow rate, buffer capacity, and pH) or the concentration of components of saliva with antimicrobial activity [3–8]. On the other hand, there are few studies on the antioxidant defence systems of saliva and their relation with oral diseases.

Moore *et al.* [9] compared the antioxidant activity of saliva collected from apparently healthy and periodontally diseased subjects. The authors reported that the antioxidant potential of saliva does not appear to be compromised in patients with periodontal disease but this may relate to the antioxidant flow from the gingival crevicular fluid.

Pereslegina [10] investigated salivary total antioxidant, catalase, superoxide dismutase, glutathione

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peroxidase, glutathione reductase, and glutathione transferase activities in different salivary glands of normal children in function with different seasons. Interestingly, major antioxidants found in saliva, total antioxidant status of saliva, and the relevance of this to caries protection have not been investigated yet in children.

The purpose of this study was to evaluate the relation between the physicochemical properties of saliva such as flow rate, buffering capacity, pH, calcium level, total protein, and total antioxidant status, and dental caries, age, and gender.

Material and methods

Eighty healthy children, aged between 7 and 15 years old, who were not taking medications and not exposed to systemic or topical fluoride during tooth development were recruited. Their informed consent was obtained, and the study was approved by the Faculty of Medicine Ethic Committee, University of Ankara.

Subjects were divided into two subgroups according to ages between 7–10 and 11–15 years and each group was composed of 40 subjects (n = 80). As gender and caries status were the selection criteria, subgroups of equal size were formed. The groups are summarized in Table 1. The presence of caries was also examined radiographically on bitewing radiographs taken from each subject. One experienced dentist examined all patients for their adherence to inclusion criteria. Caries active (CA) was defined as having at least five decayed tooth surface requiring restoration, and caries-free (CF) subjects were those without clinically detected caries: DMFS = 0. Caries status was assessed according to the World Health Organization [29] recommendations. A special diet was assigned to the subjects by a dietician 24 h before saliva collection for investigation.

Table 1. Study groups.

Gender	Age	Caries activity	*n*
Girl	7–10	Caries free	10
Girl	7–10	Caries active	10
Boy	7–10	Caries free	10
Boy	7–10	Caries active	10
Girl	11–15	Caries free	10
Girl	11–15	Caries active	10
Boy	11–15	Caries free	10
Boy	11–15	Caries active	10

Children were seen in the morning at least 2 h after eating or drinking.

Saliva samples were collected using the method described by Dawes in 1987 [11]. The saliva was collected into a preweighed tube on ice during a 5-min period. After collection, the tube was weighed again and the flow rate calculated.

Immediately after the completion of collection, the pH was measured by a manual pH meter (Hanna Instruments, Kehl am Rhein, Germany) and the buffer capacity was determined by the method of Ericsson [12] modified for smaller volumes. This method involved the addition of 0·5 mL of saliva was added to 1·5 mL of 5 mmol/L HCl. The mixture was vigorously shaken and allowed to stand for 10 min when the final pH was measured. The remaining saliva was kept at 4 °C and transported to the laboratory within 30 min and kept at −80 °C until analysed.

Measurement of total protein and calcium:

The total protein and calcium levels of the samples were measured by auto analyser (Syncron CX7, Beckman Coulter, USA). The principle of the total protein assay was based on the biuret method and included alkaline copper reagent. The proteins in saliva produce an alkaline copper–protein chelate when combined with the reagent. The resulting increase in absorbency was monitored by a detector at 545 nm. The observed rate of chelate formation was directly proportional to the total protein concentration in the sample.

The salivary calcium concentration was measured by the Arsenazo-lll method (Sigma-Aldrich, St Louis, MO, USA). The absorbency of the reagent was measured bichromatically at 650 and 700 nm immediately before and 21 s after sample addition. The change in absorbency was directly proportional to the amount of calcium in the sample.

Total antioxidant status assay:

Total salivary antioxidant activity was measured with an autoanalyser (Technicon RAXT, USA). Reactives required for assay were obtained from Randox, Krefeld, Deutschland. Total antioxidants of the samples were estimated by an adaptation of the ABTS; 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulphonate) assay, which involves the interaction of the ferrylmyoglobin radical produced from activation of methmyoglobin, with the ABTS forming the ABTS radical cation. The suppression of blue/green colour production is proportional to concentration.

All data were evaluated with nonparametric statistics (Mann–Whitney U-test), and statistically

Table 2. Mean values and their standard deviations of test groups.

Gender	Age	Caries activity	Flow rate (mL/min)	pH	Buffer capacity	Ca (mg/dL)	Total protein (g/dL)	Total antioxidant mmol/L
Girl	7–10	Caries free	0·270 ± 0·07	7·26 ± 0·45	5·09 ± 0·50	3·18 ± 1·60	0·33 ± 0·08	0·39 ± 0·08
Girl	7–10	Caries active	0·269 ± 0·05	7·18 ± 0·20	4·62 ± 0·50	2·32 ± 0·70	0·35 ± 0·09	0·45 ± 0·13
Boy	7–10	Caries free	0·379 ± 0·11	7·03 ± 0·43	5·01 ± 0·7	3·59 ± 2·30	0·35 ± 0·14	0·48 ± 0·20
Boy	7–10	Caries active	0·318 ± 0·06	7·22 ± 0·33	5·33 ± 0·70	2·39 ± 0·50	0·65 ± 0·5	0·60 ± 0·15
Girl	11–15	Caries free	0·269 ± 0·04	6·8 ± 0·38	4·70 ± 0·41	5·09 ± 0·98	0·52 ± 0·10	0·65 ± 0·16
Girl	11–15	Caries active	0·299 ± 0·07	7·37 ± 0·19	4·69 ± 0·61	3·17 ± 0·71	0·43 ± 0·43	0·57 ± 0·18
Boy	11–15	Caries free	0·330 ± 0·12	7·03 ± 0·48	4·78 ± 0·5	7·73 ± 4·6	0·45 ± 0·14	0·56 ± 0·30
Boy	11–15	Caries active	0·370 ± 0·06	7·0 ± 0·23	5·32 ± 0·80	3·01 ± 1·10	0·48 ± 0·16	0·69 ± 0·25

Ca, Calcium.

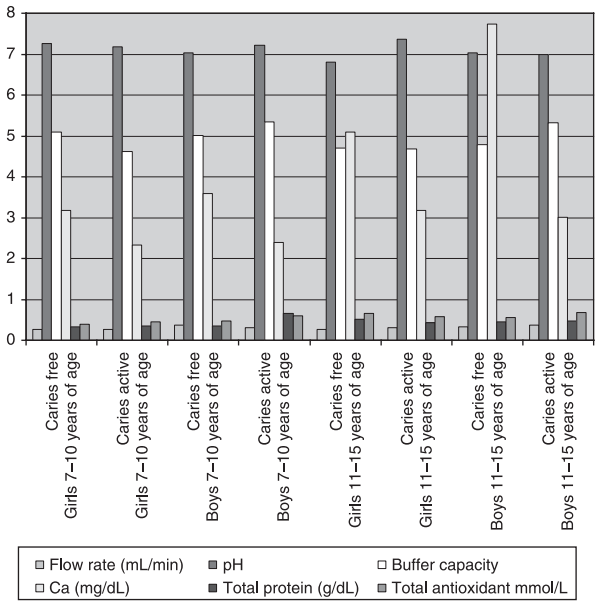

Fig. 1. Mean salivary composition values.

significant findings reported in the tables and text were confirmed. A *P*-value of less than 0·05 was considered significant. The comparison of salivary compositions of CA and CF children without considering sex and age factors were evaluated with *t*-tests. Mean salivary composition values related to caries activity, age, and gender were evaluated with Mann–Whitney *U*-tests.

Results

The mean numbers of decayed, missing, or filled surfaces for 7- to 10-year-old girls were 10·8 ± 4·1, 7- to 10-year-old boys were 10·3 ± 3·0, 11- to 15-year-old girls were 6·6 ± 1·1, and 11- to 15-year-old boys were 6·0 ± 1·2, respectively. In all groups, salivary flow rates were almost the same, whereas pH, buffer capacity, total protein, and total antioxidant values were higher in the CA group but the differences were not statistically significant ($P > 0·05$). The salivary concentration of calcium was significantly higher in the CF group ($P < 0·05$).

Mean salivary composition values of test groups are exhibited in Fig. 1. Mean values and their standard deviations of test groups are shown in Table 2. The results of Mann–Whitney *U*-tests are shown in Table 3.

Discussion

For a clinician, saliva means ‘whole saliva’, which is the fluid present in the mouth and comprises. not only pure secretions from the major and minor salivary glands but also gingival exudates, microorganisms, and their products, epithelial cells, food remnants, and also to some extent nasal exudates [5].

In this study, special care was taken to standardize the factors affecting the subjects in so far as one is able, in order to simplify the discussion and conclusion of the results. Diet, genetic factors, and environmental effects, however, should always be considered as the cause of an eventual biological variance. The age range of the subjects was kept narrow and the same diet was stipulated to all subjects before 24 h of the sample collection. Additionally, it was decided to take unstimulated saliva samples as it is preferred in determination of antioxidant defence parameters to stimulated saliva [10,13] and it is claimed that TAA is higher in unstimulated saliva [9].

No linear relationship has been found between salivary flow rate and caries activity, except when the salivary flow rate is below the theshold level [11]. In this study, also no significant correlation between

Table 3. Statistical evaluations of test groups (Mann–Whitney *U*-tests).

		Flow rate	pH	Buffer capacity	Ca	Total protein	Total antioxidants
Caries status	CA 7–10 age girls vs. CA 7–10 age girls	$U = 47$, $P = 0{\cdot}85$	$U = 22$, $P = 0{\cdot}03$	$U = 22$, $P = 0{\cdot}03$	$U = 37$, $P = 0{\cdot}35$	$U = 47$, $P = 0{\cdot}85$	$U = 37$, $P = 0{\cdot}35$
	CA 7–10 age boys vs. CF 7–10 age boys	$U = 30$, $P = 0{\cdot}14$	$U = 40$, $P = 0{\cdot}48$	$U = 35$, $P = 0{\cdot}27$	$U = 37{\cdot}5$, $P = 0{\cdot}12$	$U = 30$, $P = 0{\cdot}14$	$U = 37$, $P = 0{\cdot}35$
	CA 11–15 age girls vs. CF 11–15 age girls	$U = 39{\cdot}5$, $P = 0{\cdot}43$	$U = 55$, $P = 0{\cdot}00$	$U = 50$, $P = 1{\cdot}0$	$U = 40$, $P = 0{\cdot}00$	$U = 21$, $P = 0{\cdot}28$	$U = 36$, $P = 0{\cdot}31$
	CA 11–15 age boys vs. CF 11–15 age boys	$U = 41{\cdot}5$, $P = 0{\cdot}52$	$U = 46$, $P = 0{\cdot}79$	$U = 41{\cdot}5$, $P = 0{\cdot}35$	$U = 15{\cdot}5$, $P = 0{\cdot}068$	$U = 45{\cdot}5$, $P = 0{\cdot}73$	$U = 34{\cdot}5$, $P = 0{\cdot}24$
Age	7–10 age CF girls vs. 11–15 age CF girls	$U = 47$, $P = 0{\cdot}85$	$U = 10$, $P = 0{\cdot}00$	$U = 33{\cdot}5$, $P = 0{\cdot}21$	$U = 17{\cdot}5$, $P = 0{\cdot}01$	$U = 7{\cdot}0$, $P = 0{\cdot}00$	$U = 12$, $P = 0{\cdot}00$
	7–10 age CA girls vs. 11–15 age CA girls	$U = 37{\cdot}5$, $P = 0{\cdot}35$	$U = 22{\cdot}5$, $P = 0{\cdot}03$	$U = 49$, $P = 0{\cdot}97$	$U = 19$, $P = 0{\cdot}01$	$U = 0{\cdot}24$, $P = 0{\cdot}05$	$U = 27{\cdot}5$, $P = 0{\cdot}08$
	7–10 age CF boys vs. 11–15 age CF boys	$U = 41$, $P = 0{\cdot}52$	$U = 49{\cdot}5$, $P = 0{\cdot}97$	$U = 43{\cdot}5$, $P = 0{\cdot}63$	$U = 20{\cdot}5$, $P = 0{\cdot}02$	$U = 31{\cdot}5$, $P = 0{\cdot}16$	$U = 44$, $P = 0{\cdot}68$
	7–10 age CA boys vs. 11–15 age CA boys	$U = 27$, $P = 0{\cdot}08$	$U = 27{\cdot}5$, $P = 0{\cdot}08$	$U = 48$, $P = 0{\cdot}91$	$U = 37$, $P = 0{\cdot}35$	$U = 47{\cdot}5$, $P = 0{\cdot}83$	$U = 37{\cdot}5$, $P = 0{\cdot}35$
Gender	7–10 age CA girls vs. 7–10 age CA boys	$U = 27$, $P = 0{\cdot}08$	$U = 25$, $P = 1{\cdot}0$	$U = 18{\cdot}5$, $P = 0{\cdot}01$	$U = 43{\cdot}5$, $P = 0{\cdot}69$	$U = 28{\cdot}5$, $P = 0{\cdot}1$	$U = 23$, $P = 0{\cdot}04$
	11–15 age CA girls vs. 11–15 age CA boys	$U = 22{\cdot}5$, $P = 0{\cdot}03$	$U = 9$, $P = 0{\cdot}00$	$U = 23$, $P = 0{\cdot}04$	$U = 37$, $P = 0{\cdot}35$	$U = 37$, $P = 0{\cdot}35$	$U = 33$, $P = 0{\cdot}21$
	7–10 age CF girls vs. 7–10 age CF boys	$U = 21$, $P = 0{\cdot}02$	$U = 27$, $P = 0{\cdot}08$	$U = 44$, $P = 0{\cdot}68$	$U = 48$, $P = 0{\cdot}91$	$U = 45{\cdot}5$, $P = 0{\cdot}73$	$U = 36$, $P = 0{\cdot}31$
	11–15 age CF girls vs. 11–15 age CF boys	$U = 39{\cdot}5$, $P = 0{\cdot}43$	$U = 33{\cdot}5$, $P = 0{\cdot}21$	$U = 46{\cdot}5$, $P = 0{\cdot}79$	$U = 40$, $P = 0{\cdot}48$	$U = 32{\cdot}5$, $P = 0{\cdot}19$	$U = 33{\cdot}5$, $P = 0{\cdot}21$

CA, caries active; CF, caries free; Ca, calcium.

caries activity and salivary flow rate were established. The effect of age, gender, and salivary flow rate is not entirely clear. Some researchers have noted no effect [14,15], whereas others have reported lower rates in females [16–19]. In this study, salivary flow rates were higher in boys compared with girls but only the differences between 7- and 10-year-old CF children (10 subjects), and the differences between 11- and 15-year-old CA children (10 subjects) were statistically significant.

Saliva is effective in helping to maintain a neutral pH in the oral cavity and on swallowing, in the oesophagus as well [20]. In this study, salivary pH values were higher in the CA group. Whereas in 7- to 10-year-old girls, pH values were higher in the CF group (10 subjects) than in the CA girls; in the 11 to 15 years of age group, pH values were significantly higher in CA girls. When the changes with age were considered, significant differences were found between girls aged 7 and 10 and 11 to 15 years. While pH values decreased with age in the CF group, in the CA group pH values increased with age. In the 11 to 15 years of age group, significant differences were found between pH values of CA girls and boys. As a result, it can be concluded that there were no correlations between pH values and caries activity, age, or gender. As stated below, individual and environmental variations could be an important cause of the results.

Most investigators have reported, as reviewed by Ericsson [12], that the salivary buffering capacity has an inverse relationship with human caries incidence. Further, there is evidence that different foods, such as dietary proteins and carbohydrates, can affect the salivary buffering capacity [21]. Consistently in this study, in the 7 to 10 years of age CA groups, buffer capacity values were significantly lower than in the CF group. There were no significant differences when the groups were compared related to age, except only in CA groups where buffer capacity values were significantly higher in boys than in girls. No correlation was found between buffer capacity and caries activity, age, and gender.

Although in former studies, no significant correlations were found between salivary calcium concentration and caries activity, in this study, salivary calcium values were significantly higher in CF children than in CA children in both gender and age groups. Salivary calcium values increased with age in all groups except in 11- to 15-year-old boys. In all groups, salivary calcium values were higher in boys than in girls except with the 11 to 15 years of age group in which CA boys showed lower values than CA girls. These results indicate that a relation exists between salivary calcium levels and caries activity.

Interest in saliva proteins stems from their role in host defence mechanisms: the protection of oral soft and hard tissues [20]. Our finding that the saliva protein level is increased with age is consistent with the literature [22] and is not surprising as the selected age group represent a rapid growth phase [23–25]. Protein deficiency influences markedly the composition of whole saliva in man [23,24,26,27].

Although quantitative studies of salivary composition and caries activity have been inconclusive, there is evidence that similar proteins in saliva from CA and CF persons may have different levels of biological activity [28]. Although they were not statistically significant, the increase in protein level in CA children of both age groups, and conformity of this increase with total antioxidant values is quite remarkable, as it is very well known that lots of components in protein structure exist under total antioxidants [1]. These findings are also consistent with previous reports claiming that total protein level is higher in those with dental caries [28]. The higher total antioxidant values in CA children can be attributed to elevated protein levels. In a previous study [9], it has been suggested that the major antioxidant in saliva was urate, thus it can be concluded that salivary antioxidant levels must be in a linear association with total protein levels. That was in accordance with the findings of this study.

Interestingly, all of the results showing differences were those from CA girls in 11 to 15 years of age group. That is to say, total protein and total antioxidant were found to be decreased (not statistically significant) and pH values of this group significantly increased. These ages (10–15 years) are known to be related with increase of sexual hormone levels especially in girls. Thus, it can be discussed

What this paper adds

- Total protein and total antioxidant levels in saliva increased with increasing caries activity.
- Calcium concentrations were higher in caries-free children and increased with age.

Why this paper is important for paediatric dentists

- This paper demonstrates salivary factors which may in future prove to be useful measures of caries activity in children and allow dentists to target preventive measures appropriately.

here that changes on the composition of total protein and total antioxidant might be because of pH elevation in this group. So it must be pointed out here that hormonal relationship with these parameters of the saliva should be investigated in detail.

Conclusion

In general, total protein and total antioxidant in saliva were increased with caries activity.

Ca concentrations in saliva were higher in caries-free children. In addition, Ca concentration increased with age. Thus, it can be concluded that a linear association exists between Ca concentration age and caries activity.

More clinical and laboratory studies are needed to determine the exact relationship between the physicochemical properties of saliva such as flow rate, buffering capacity, pH, calcium level, total protein, total antioxidant status, and dental caries, age, and gender.

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