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Salivary flow rate, total protein and pH in caries-free children and adolescents aged between 5 and 18 years

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Keywords

saliva, flow rate, pH, total protein, developmental age

Summary

Introduction. Data regarding salivary flow and the levels of salivary components in developmental age are scarce and not fully consistent.
Aim. The aim of the study was to compare unstimulated mixed saliva flow rate, pH and total protein in children aged between 5 and 18 years to obtain information on the functional maturation of salivary glands during the developmental period.
Material and methods. A total of 90 children and adolescents (both sexes) aged between 5 and 18 years were examined. All subjects were caries-free (ICDA II score zero). Unstimulated mixed saliva was sampled from all patients to assess pH, total protein and flow rate. The subjects were divided into age groups 5-6, 13-14 and 18 years.
The study was approved by the Bioethics Committee of the University (No. Nr KB-335/2013).
Results. Significantly lower salivary flow rates were observed in 5-6 year olds vs. 13-14 and 18-year-olds. In contrast, pH values were significantly higher in the youngest group compared to older age groups. Total protein was the lowest in 5-6 year olds, higher in 13-14 year olds and the highest at the age of 18 years (significant difference between age groups of 5-6 and 18 years). A decreasing trend in pH values and an increasing one in protein levels were observed between the age groups. Considering the entire group of subjects, a positive correlation between age and salivary flow rate and protein levels, and a negative correlation with pH were found. Moreover, pH and protein levels decreased with increasing salivary flow.
Conclusions. Unstimulated mixed saliva flow rate and total protein increase, while pH levels decrease between the ages of 5 to 18 years.

Introduction

Mixed or total saliva is a mixture of oral secretions, which come into direct contact with oral anatomical structures. It is a natural oral environment for hard and soft tissue exposure to external environmental factors and interactions between tissues, food, microbes and air. A variety of organic and mineral components contained in saliva allow for the normal course of multiple processes maintaining a healthy oral ecosystem (1, 2). Saliva is produced mainly by three paired large salivary glands, i.e. parotid, sublingual and submandibular glands, as well as, to a minor extent, by multiple (400-1000) small glands found in the oral mucosa. Under physiological conditions, the total daily volume of oral secretions ranges between 0.5 to 1 L in adults, including 80% of saliva stimulated by food. Each type of salivary gland produces secretion with a specific composition and properties, which depend on a number of factors,

including diseases and pharmacotherapy (3-6). The major salivary glands produce about 90% of the total salivary volume. The secretions produced under stimulated conditions in parotid glands, which are the largest salivary glands (serous glands), constitute a thin aqueous liquid high in α-amylase and low in organic components and glycoproteins, contributing to about 53% of total saliva (7). Under unstimulated conditions, the amount of produced saliva is significantly lower, accounting for about 20-30% (1). The submandibular gland (SMG) is the second largest gland (8), which produces serous/mucous secretions. The gland produces less than a half of total saliva under stimulated conditions and 1/3 of total saliva under unstimulated conditions (8). Dense and viscous serous/mucous secretion produced by the sublingual glands, both stimulated and unstimulated, accounts for only a small proportion of total salivary volume (1, 8). Minor salivary glands produce mucous saliva high in proteins, which accounts for about 10% of total saliva (1, 8). Normal unstimulated and stimulated salivary flow rate is about 0.25-0.35 mL/min and 1-3 mL/min, respectively. Hyposalivation, i.e. reduced salivary flow, is defined as unstimulated salivary flow rate < 0.1 mL/min and stimulated salivary flow rate < 0.5-0.7 mL/min (1, 9-11). Salivary volume depends, among other things, on the quantity and quality of consumed foods, body hydration, emotional stimuli, age and sex (12, 13). Secretion of saliva follows a circadian rhythm. During sleep, salivary glands produce only about 2-10% of total daily volume, with submandibular and sublingual contributions of about 80 and 20%, respectively, and with arrested secretion in the parotid glands. Salivary flow increases by about 25-30% in the morning. Minor salivary glands do not follow a circadian rhythm, but maintain a steady level of secretion (14, 15). In humans, major salivary glands arise from a thickening of the oral ectoderm at around 4 to 6 weeks of foetal life for parotid glands, at the end of week 6 for submandibular glands, and 7-8 weeks for the sublingual gland. Minor salivary glands arise from ectodermal and endodermal thickening at the end of the 12th week. Further development involves complex interactions between epithelial cells and the adjacent mesenchymal cells, which induces and controls morphogenesis and salivary gland cell differentiation (16). At 16 weeks of gestation, the submandibular gland starts the production of serous secretions, the production of which is reduced at 28 weeks. The parotid gland begins to secrete at 18 weeks of gestation (17). It is assumed that salivary glands are functionally capable of secreting saliva already at the time of birth (18). However, studies indicate that age-related quantitative and qualitative changes in saliva are particularly pronounced in older patients compared to young individuals (19-21). Data regarding salivary flow and the levels of salivary components in developmental age are scarce and not fully consistent.

Aim

The aim of the study was to compare salivary flow rate, pH and total protein in children and adolescents to obtain information on the functional maturation of salivary glands during the developmental period.

Material and methods

Non-cavitated and cavitated caries-free children and adolescents (classified based on the ICDAS II, code 0) were randomly selected and examined. A total of 90 subjects of both sexes were included in the study. The participants were classified into 3 age groups: 5-6, 13-14 and 18 years. Inclusion criteria were as follows: age between 5 and 6 years, between 13 and 14 years, and 18 years, full dentition with a code 0 in ICDAS II, no chronic systemic diseases or pharmacotherapy, written consent of parent/legal guardian/18-year-old patient, and patient's cooperation. Failure to meet one of the above inclusion criteria was the exclusion criterion. Clinical assessment of oral health was performed by two independent researchers (following calibration), with 90% conformity of assessment. Unstimulated mixed saliva was sampled in the morning, at least 1.5 hrs after a meal or about 4 mL of beverage. While collecting saliva, the subjects were placed in a sitting position with the head tilted and the mouth open, and were asked to let saliva gather on the bottom of their mouth and spit into calibrated tubes placed in ice. The time needed to collect saliva was recorded and salivary volume was measured to calculate salivary flow rate (mL/min). Salivary samples were then centrifuged at 3,500 rpm for 10 minutes. The obtained supernatants were used to determine salivary pH (pH-metric method using the ESAgP-301W type combined electrode connected to the pH/Ion Meter CPI-551 Microcomputer) and total protein using the Lowry's micromethod (22) based on measuring the content of tryptophan and tyrosine residues in the protein using the Folin-Ciocalteu reagent (phosphomolybdate and phosphotungstate), comparing the measured absorbance of the sample with a standard curve for bovine albumin; protein levels were expressed in mg/mL. Statistica 12.0 (StatSoft, Poland) was used for statistical analysis, using the Kolmogorow-Smirnow test to assess normal distribution of variables, followed by Tukey's test. A p-value ≤ 0.05 was considered statistically significant. The study was approved by the Bioethics Committee of the University (No. Nr KB-335/2013).

Results

Significantly lower salivary flow was found in 5-6 year olds compared to those aged 13-14 and 18 years (fig. 1). Salivary pH was significantly higher in the youngest age group compared to older groups. A linear downward trend in mean pH levels was observed between the study groups (fig. 2). Total protein levels were significantly lower

in 5-6 year olds, increased in 13-14 year olds, to reach peak values in 18-year-olds (significant difference between 5-6 year olds and 18-year-olds) (tab. 1). Furthermore, a linear upward trend in mean protein levels was found between the study groups (fig. 3).

Considering the entire study group, it was found that unstimulated salivary flow rate and total protein significantly increased, whereas salivary pH decreased with age. Furthermore, pH and salivary proteins decreased with increasing unstimulated salivary flow (tab. 2).

Discussion

Unstimulated salivary flow and salivary content of appropriate levels of biochemical components are important for oral health. Saliva has many important functions. Salivary mucins and proline-rich glycoproteins (PRGs) contribute to lubricating effects of saliva, ensuring protection (reducing the effects of mechanical, chemical and thermal mucosal damage – mucins), by wetting the mucous membrane and teeth as well as by the flow itself, which helps remove bacteria, products of their metabolism and residual food (23). High salivary content of water and mucins facilitates phonation, bolus formation, swallowing and chewing. The PRG-albumin complex is another lubricant covering the oral mucosa and reducing friction between food bolus and teeth (23).

A number of reactions involved in taste perception occur upon salivary effects on mucosal chemoreceptors in the oral cavity (24).

Saliva contains multiple enzymes, such as α-amylase, phosphatases and esterases. Alpha-amylase, which is synthesised mainly in the parotid glands, initiates digestion of extrinsic α-glycans, which are later converted to maltose (a disaccharide), which breaks down into glucose. The resulting monosaccharides are either metabolised by bacteria into acids or give rise to bacterial polysaccharides (25-27). Saliva helps maintain mucosal and periodontal

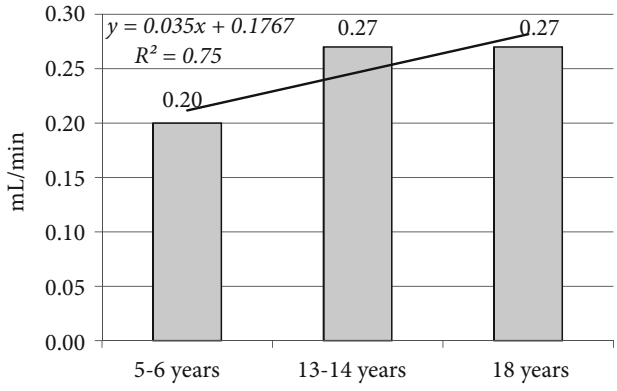

Fig. 1. Age-related trend in salivary flow rate

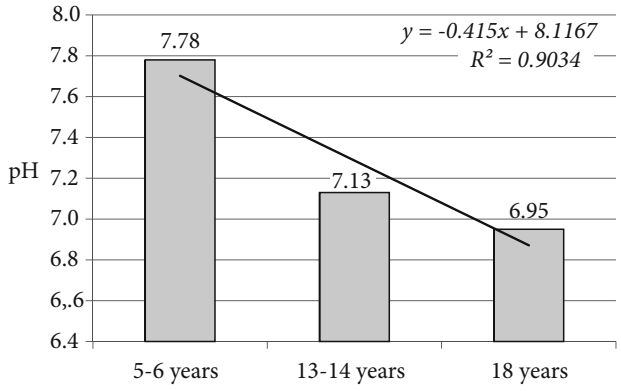

Fig. 2. Age-related trend in salivary pH

Tab. 1. Salivary parameters in different age groups of caries-free patients

Parameter	Age			Significance of differences – p-value
	5-6 years	13-14 years	18 years	
	x ± SD	x ± SD	x ± SD	
Salivary flow rate mL/min	0.20 ± 0.07[a, b]	0.27 ± 0.11[a, c]	0.27 ± 0.08[b, c]	[a-a]p = 0.0102* [b-b]p = 0.0055* [c-c]p > 0.05 ns
pH	7.78 ± 0.55[a-c]	7.13 ± 0.38[a, c]	6.95 ± 0.48[b]	[a-a]p = 0.0000* [b-b]p = 0.0000* [c-c]ns
Total protein mg/mL	0.65 ± 0.17[a, b]	0.86 ± 0.28[b, c]	1.09 ± 0.48[a, c]	[a-a]p = 0.0002* [b-b]ns [c-c]ns

*statistically significant; ns – not significant

The compared pairs are marked with the same letters.
For example: the difference between 5-6 years (a) and 13-14 years (a) statistically significant (*, p = 0.0102); difference between 5-6 years (b) and 18 years (b) statistically significant (*, p = 0.055); the difference between 13-14 years (c) and 18 years (c) not statistically significant (p> 0.05).

Tab. 2. Correlation coefficients between the analysed salivary parameters and patient's age

Parameters	Age	Salivary flow rate mL/min	pH
Salivary flow rate mL/min	r = 0.338 p = 0.001*		
pH	r = -0.576 p = 0.0000*	r = -0.373 p = 0.0002*	
Total protein mg/mL	r = 0.475 p = 0.0000*	r = -0.440 p = 0.0000*	r = 0.137 p = 0.196

*statistically significantly dependent correlation coefficient

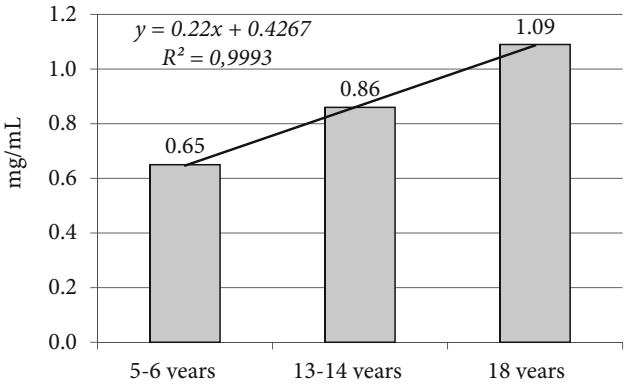

Fig. 3. Age-related trend in salivary total protein

integrity in the oral cavity (28), as well as contributes to mucosal wound healing (29, 30), remineralisation (31, 32) and maintaining oral pH (33).

Age-related changes in salivary composition may result from the physiological development of salivary glands. It has been postulated that although human salivary glands develop already in prenatal life, their further functional development continues in childhood and ends in adolescence (34). A number of studies indicate lower unstimulated mixed salivary flow in children compared to adults, as well as increasing salivary flow with age (35-38). Wu et al. (37) observed an increased unstimulated salivary flow in school children compared to preschool children. Our findings support this thesis for unstimulated saliva. We showed a significant increase in unstimulated mixed salivary flow rate between 5-6 year olds and 13-14 year olds and no further increase between 13-14 year olds and 18-year-olds. This may, to some extent, support the thesis presented by Crossner (34), who concluded, based on the assessment of stimulated saliva, that salivary glands reach full maturity at the age of 15 years. Furthermore, a positive co-variability of salivary flow rate and age was found for the entire study group. However, Tulunoglu et al. (39) fund no such a relationship among patients aged between 7 and 15 years, and neither did Forcella et al. (40) for 6 to 15 year-olds or Wu et al. (37) for 3 to 14 year-olds. This may be due to the technique for salivary collection, and thus the accuracy of measurements.

Our study showed a significant negative correlation between salivary pH and age as opposed to Piróg et al. (41) and Forcella et al. (40), who used Saliva Check Buffer for pH measurement. However, we found a positive correlation between age and protein levels, which corresponds to the findings presented by Wu et al. (37).

Hyyppä et al. (42) assessed total protein in unstimulated saliva in toothless children aged between 2 and 6 months (mean age 4.3 months) and in the same children at the age of 12 to 19 months (mean age 12.7 months) with a few erupted teeth and in adults aged between 21 and 31 years (mean age 23.3 years). The authors observed similar protein levels in children with no or a few teeth, which were significantly lower compared to adults. In their study in 3-14 year olds, without considering dental caries, Wu et al. (37) demonstrated the highest protein levels in mixed unstimulated saliva in 12-14 year olds, and the lowest levels in 3-5 year olds. Similarly, we observed the lowest protein levels in 5-6 year olds, and the highest in 18-year-olds. Furthermore, a positive correlation between age and protein levels was reported for the entire study group. We also found a positive co-variability for salivary flow rate and pH, which corresponds to the findings presented by Forcella et al. (40).

Conclusions

Unstimulated salivary flow rate and total protein levels increase, while unstimulated mixed salivary pH values decrease between 5 and 18 years of age.

Conflict of interest

None

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References

1. Humphrey SP, Williamson RT: A review of saliva: normal composition, flow and function. J Prosthet Dent 2001; 85: 162-169.
2. Falcão DP, da Mota LM, Pires AL et al.: Sialometry: aspects of clinical interest. Rev Bras Reumatol 2013; 53: 525-531.
3. Drobitch RK, Svensson CK: Therapeutic drug monitoring in saliva. An update. Clin Pharmacokinet 1992; 23: 365-379.
4. Forde MD, Koka S, Eckert SE et al.: Systematic assessments utilizing saliva: Part 1 General Considerations and Current Assessments. Int J Prosthodont 2006; 19: 43-52.
5. Sreebny LM: Saliva in health and disease: an appraisal and update. Int Dent J 2000; 50: 140-161.
6. Murray Thomson W, Poulton R, Broadbent JM et al.: Xerostomia and medications among 32-year-old. Acta Odontol Scand 2006; 64(4): 249-254.
7. Proctor GB: The physiology of salivary secretion. Periodontol 2000 2016; 70(1): 11-25.
8. Silvers AR, Som PM: Salivary Glands. Radiol Clin North Am 1998; 36: 941-966.
9. Bergdahl M: Salivary flow rate and oral complaints in adult dental patients. Community Dent Oral Epidemiol 2000; 28(1): 59-66.
10. Paszyńska E: Wybrane czynniki wpływające na wydzielanie i skład śliny – omówienie aktualnego piśmiennictwa. Dental Forum 2005; 1: 86-90.
11. Kaczmarek U: Suchość jamy ustnej – etiologia, częstość występowania i rozpoznanie na podstawie piśmiennictwa. Czas Stomatol 2007; LX(1): 20-31.
12. Szydlarska D, Grzesiuk W, Kupstas A, Bar-Andziak E: Ślina jako materiał diagnostyczny. Forum Medycyny Rodzinnej 2008; 2(6): 454-464.
13. Mandel ID: The functions of saliva. J Dent Res 1987; 66: 623-627.
14. Nederfors T, Dahlof C: Effects of the beta-adrenoceptor antagonists atenolol and propanolol on human whole saliva flow rate and composition. Arch Oral Biol 1992; 37(7): 579-584.
15. Rantonen PJ, Meurman JH: Viscosity of whole saliva. Acta Odontol Scand 1998; 56(4): 210-214.
16. de Paula F, Teshima THN, Hsieh R et al.: Overview of human salivary glands: highlights of morphology and developing process. Anat Rec (Hoboken) 2017; 300(7): 1180-1188.
17. Som PM, Miletich I: The Embryology of the Salivary Glands: An Update. Neurographics 2015; 5(4): 167-177.
18. Holmberg KV, Matthew P: Hoffman MP: Anatomy, biogenesis and regeneration of salivary glands. Monogr Oral Sci 2014; 24: 1-13.
19. Heintze U, Birkhed D, Bjorn H: Secretion rate and buffer effect of resting and stimulated whole saliva as a function of age and sex. Swed Dent J 1983; 7(6): 227-238.
20. Ship JA, Pillemer SR, Baum BJ: Xerostomia and the geriatric patient. J Am Geriatr Soc 2002; 50(3): 535-543.
21. Sreebny LM, Valdini A: Xerostomia. Part I: Relationship to other oral symptoms and salivary gland hypofunction. Oral Surg Oral Med Oral Pathol 1988; 66(4): 451-458.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193(1): 265-275.
23. Jankowska AK, Waszkiel D, Kowalczyk A: Ślina jako główny składnik ekosystemu jamy ustnej. Część I. Mechanizm wydzielania i funkcje. Wiad Lekarskie 2007; LX(3-4): 148-154.
24. Shatzman AR, Henkin RI: Gustin concentration changes relative to salivary zinc and taste in human. Proc Natl Acad Sci USA 1981; 78: 3867-3874.
25. Kaczmarek U: O właściwościach amylaz ślinowych. Wrocł Stomat 1990: 201-208.
26. Wesley-Hadzija B, Pigon H: Effect of diet in West Africa on human salivary amylase activity. Archs Oral Biol 1972; 17: 1415-1418.
27. Makinen KK, Scheinin A: Turku sugar studies. VII. Principal biochemical findings on whole saliva and plaque. Acta Odont Scand 1976; 34: 241-248.
28. Hakkinen L, Uitto V, Larjava H: Cell biology of gingival wound healing. Periodontology 2000; 24: 127-152.
29. Pytko-Polończyk J: Rola epidermalnego czynnika wzrostu i ślinianek w procesie gojenia owrzodzeń błony śluzowej jamy ustnej i żołądka. Czas Stomat 1997; 50(9): 579-587.

30. Bernardi G, Giro M, Gallard C: Chromatography of purification and proteins on hydroxyapatite columns: some new developments. Biochem Biophys Acta 1972; 278: 409-420.
31. Adamczyk E: Rola śliny w powstawaniu płytki nazębnej i płytki protez. Protet Stomatol 1992; 42(5): 153-154.
32. Llena-Puy C: The role of saliva in maintaining oral health and as an aid to diagnosis. Med Oral Patol Oral Cir Bucal 2006; 11(5): E449-E455.
33. Edgar M, Dawes C, O'Mullane D: Saliva and oral health. BDJ Books, London 2004: 1-136.
34. Crossner CG: Salivary flow rate in children and adolescents. Swed Dent J 1984; 6(8): 271-276.
35. Watanabe S, Dawes C: Salivary flow rates and salivary film thickness in five-year-old children. J Dent Res 1990; 69(5): 1150-1153.
36. Bretz WA, do Valle EV, Jacobson JJ et al.: Unstimulated salivary flow rates of young children. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001; 91(5): 541-545.
37. Wu KP, Ke J-Y, Chung C-Y et al.: Relationship between unstimulated salivary flow rate and saliva composition of healthy children in Taiwan. Chang Gung Med J 2008; 31: 281-286.
38. Torres SR, Nucci M, Milanos E et al.: Variations of salivary flow rates in Brazilian school children. Braz Oral Res 2006; 20(1): 8-12.
39. Tulunoglu O, Demirtas S, Tulunoglu I: Total antioxidant levels of saliva in children related to caries, age, and gender. Int J Paediatr Dent 2006; 16(3): 186-191.
40. Forcella L, Filippi C, Waltimo T et al.: Measurement of unstimulated salivary flow rate in healthy children aged 6 to 15 years. Swiss Dent J 2018; 128(12): 962-967.
41. Piróg A, Kalińska A, Gozdowski D, Olczak-Kowalczyk D: Influence of physicochemical parameters of saliva on dentition, gingiva and oral mucosa in healthy children. J Stoma 2013; 66(2): 154-169.
42. Hyyppä T, Karhuvaara L, Tenovuo J et al.: A longitudinal factors in whole saliva of human infants: a longitudinal study. Pediatr Dent 1989; 11(1): 30-36.

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