Relationship between Unstimulated Salivary Flow Rate and Saliva Composition of Healthy Children in Taiwan

Katie P. Wu, MD; Jyh-Yuh Ke, MD; Chia-Ying Chung, MD; Chia-Ling Chen, MD, PhD; Tsong-Long Hwang[2], PhD; Ming-Yen Chou[1], DDS, MD; Alice M. K. Wong, MD; Ching-Fang Hu, MD; Yu-Cheng Lee, MD

Background: Saliva is one of the most important factors in regulating oral health, with flow rate and composition changing throughout development and during disease. In view of the shortage of data, the present study aimed to shed light on the relationship between unstimulated salivary flow rate and saliva composition of healthy children in Taiwan.

Methods: Forty-four normal, healthy children from 3-14 years of age were divided into three age groups: pre-school, elementary school and junior-high school. All participants received salivary flow rate, pH and saliva composition analysis under unstimulated conditions. One-way ANOVA and Pearson's correlation were used. Statistical significance was set at $p < 0.05$.

Results: Our results suggest that, under unstimulated conditions, the salivary flow rate of the elementary school group was greater than that of the pre-school group ($p < 0.05$). No difference in pH was found among the three groups. Intergroup salivary calcium, phosphorus and amylase did not reach statistical difference. As the flow rate increased, the pH increased ($r = 0.364$, $p < 0.05$) but the protein level decreased ($r = -0.473$, $p < 0.05$). In addition, salivary protein was positively correlated to age ($r = 0.479$, $p < 0.05$) and negatively correlated to pH ($r = -0.361$, $p < 0.01$).

Conclusion: Age-related increase in the unstimulated salivary flow rate of pre-school and elementary school groups was noted. As the flow rate increased, the pH increased but the protein level decreased. The information obtained may serve as reference values for the growing interest in saliva as a diagnostic tool, especially monitoring those with neurological or oral motor dysfunction.

(Chang Gung Med J 2008;31:281-6)

Key words: children, unstimulated salivary flow rate, composition, pH

Saliva plays a critical role in maintaining oral homeostasis; it modulates the ecosystem through lubrication of the alimentary bolus, protection against microorganisms, buffer and repair of the oral mucosa, and helps in dental remineralization. However, it receives little attention until quantity or

From the Department of Physical Medicine and Rehabilitation; [1]Department of Dentistry, Chang Gung Memorial Hospital, Taipei, Chang Gung University College of Medicine, Taoyuan, Taiwan; [2]Graduate Institute of Natural Products, College of Medicine, Chang Gung University, Taoyuan, Taiwan.
Received: Jul. 25, 2006; Accepted: Aug. 2, 2007
Correspondence to: Dr. Chia-Ling Chen, Department of Rehabilitation Medicine, Chang Gung Memorial Hospital. No. 5, Fusing St., Gueishan Township, Taoyuan County 333, Taiwan (R.O.C.) Tel.: 886-3-3281200 ext. 3846; Fax: 886-3-3281320; E-mail: ccl1374@cgmh.org.tw

quality is diminished.[1] Saliva is composed of organic, inorganic and macromolecules. Calcium and phosphate ions are both involved in calculus formation and in protecting against caries. Salivary proteins have protective antimicrobial, lubricative and digestive functions, which provide a barrier between toxins and oral soft tissues, and modulate salivary calcium and phosphate chemistry.[2] Amylase is related to digestive function and, to a certain extent, it may play a role in modulating the adhesion of bacterial species to the teeth.[3]

On average, unstimulated salivary flow rate is 0.3 mL/min in the general population.[1] The concentration of various components of saliva is markedly affected by variation in flow rate.[4-6] The variation of salivary constituents over time may reflect hormonal factors, external influences and systemic conditions.[5,7] Previous investigations have shown that the salivary flow rate fluctuates with the circadian cycle.[4,7] It has been suggested that the unstimulated flow rate may be at its maximum in the mid-afternoon.[8,9] Moreover, variation of unstimulated whole saliva flow rate over different time-spans and at different times of the year yield changes in flow rate.[7] To avoid the circadian effect, obtaining saliva at the same time of day is essential.

Evidence has suggested that there is great variation in individual salivary flow rates and that individualized measurements should be recorded as a base reference after the age of 15 years.[1,10] Saliva has been detected in babies soon after birth.[4,11] The flow rate appears to increase up to the age of 5 years.[4] The unstimulated salivary flow rate ranges from 0.22-0.82 ml/min in children to 0.33-1.42 ml/min in adults.[1,10-15] The resting salivary flow rate increases throughout childhood and may reflect a developmental process: a decrease during aging may suggest a consequence of parenchymal atrophy.[15,16]

Previous literature shows the concentration of various components of saliva is markedly affected by variations in flow rate.[4,5] For example, as the flow rate of the parotid gland increases above the unstimulated rate, pH increases but potassium, calcium, phosphate and protein decrease in adults.[4] At higher flow rates, protein, pH and phosphate decrease, and potassium remains unchanged in adults.[4] Controversial findings were also reported by Siqueira, indicating that the potassium ion is not influenced by flow rate in children aged 6-10 years.[17] The differences in ionic composition suggest further investigation is required.

Reports of unstimulated salivary flow rates in healthy young children have been scarce and a growing interest in saliva as a diagnostic tool warrants further investigation. To the best of our knowledge, no previous studies have been devoted to this area of interest. The purpose of the present study was to provide information pertaining to unstimulated salivary flow rates and saliva composition in healthy children aged 3-14 years in Taiwan.

METHODS

Subjects

Forty-four normal, healthy children ranging from 3-14 years of age were selected from schools in Tao-Yuan County in June 2004. Eighteen girls and twenty-six boys with an average age of 7 years were stratified into three groups: the pre-school group (3-5 years), the elementary school group (6-11 years) and the junior-high school group (12-14 years). Exclusion criteria included developmental delay, auditory or visual dysfunction, known neurological diseases and drug use. All participants received clinical and laboratory assessments, and basic information included age, gender and medical history. Signed informed consent forms were obtained from the custodial parent or guardian of the subject. Salivary flow rate, pH and salivary composition under unstimulated conditions were analyzed.

Laboratory assessment

Saliva collection and measurements

To minimize the effect of circadian rhythms, all whole saliva samples were collected one hour after lunch for the unstimulated condition. The child was seated in a well-ventilated and well-lit room. The head was kept at 45 degrees flexion with one hand holding onto a pre-weighed disposable cup for 2 minutes or held by carers, in a calm atmosphere to simulate unstimulated conditions. The saliva was allowed to drip into the cup held to the lower lip. For each trial, the collection continued for 2 minutes but if the saliva sample was insufficient within 2 minutes, the collection was continued until 3 ml of saliva per subject was obtained. An electronic device with an accuracy of 0.1 gram (multi-function, portable pocket scale, Taiwan) was used to weigh the cup to calculate the salivary flow rate (ml/min). Immediately after collection of the specimen, the sample was frozen in dry ice and transported to the laboratory for further pH analysis.

Saliva composition analysis

The fresh saliva specimen was kept in an ice bucket and sent to the laboratory for analysis within one hour. The pH reading was recorded with a pH-meter (Suntex SP-701, Taipei, Taiwan) and the protein was quantified by a bicinchoninic acid (BCA)-protein assay kit in μg/ml. The calcium, phosphorus and potassium ionic composition (Hitachi 7600-210, Tokyo, Japan) and the enzyme amylase level (VITROS 250, Johnson & Johnson Company, U.S.A.) were also examined.

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS for Windows, version 10.0). One-way ANOVA was used to analyze the differences in salivary flow rate and saliva composition among the three groups. Dunnett's T3 test was used for *post hoc* comparison. Pearson's correlation was used to investigate the associations between salivary flow rate, pH, protein and age. Statistical significance was set at $p < 0.05$.

RESULTS

The unstimulated salivary flow rate for the pre-school group ranged from 0.75 ml/min for the lowest and 1.42 m/l/min for the highest. The rate for the elementary school group ranged from 1.36 m/l/min to 2.03 m/l/min, and the junior-high school group had a range of 0.96 m/l/min to 1.53 m/l/min. The elementary school group produced higher salivary flow rates than the pre-school group ($p < 0.05$, Table 1).

In reference to the pH value, we obtained 7.1 ± 0.28 for the pre-school group, 7.44 ± 0.30 for the elementary school group and 7.24 ± 0.32 for the junior-high school group (Table 1). The inter-group pH value was not significantly different among the three groups.

For the macromolecules, the junior-high school group had higher protein content in unstimulated saliva than the elementary school group ($p < 0.01$, Table 1) and the pre-school group ($p < 0.05$), while the amylase analysis showed no inter-group difference. With respect to the analysis of inorganic components, the potassium content of both the elementary school and the junior-high school groups was significantly greater than that of the pre-school group ($p < 0.05$). The salivary calcium and phosphorus levels did not reach statistical differences among the three age groups.

Pearson's correlation analysis showed salivary protein was positively correlated to age ($r = 0.479$, 95% CI: 0.216-0.828, $p < 0.05$, Table 2). The unstimulated salivary flow rate was also positively correlated to pH ($r = 0.364$, 95% CI: 0.075-0.688, $p < 0.05$, Table 2). Conversely, the protein content was negatively correlated to the salivary flow rate ($r = -0.473$, 95% CI: −0.820 to −0.208, $p < 0.05$, Table 2) and the

Table 1. Salivary Flow Rate, pH, Protein and Composition under Unstimulated Conditions

	3~5 years (Group A) n = 12	6~11 years (Group B) n = 16	12~14 years (Group C) n = 16	*p* value (between) Age	Sex
Flow rate (ml/min)	1.09 ± 0.52	1.69 ± 0.63	1.25 ± 0.54	AB†	0.121
pH	7.17 ± 0.28	7.44 ± 0.30	7.24 ± 0.22	0.035‡	0.055
Macromolecules					
Protein (μg/ml)	582.2 ± 261.1	412.2 ± 153.8	905.8 ± 264.7	AC† BC*	0.511
Amylase (U/L)	631.7 ± 254.4	815.8 ± 526.8	1047.4 ± 471.2	0.220	0.499
Inorganic component					
K mg/dl	16.93 ± 1.44	20.92 ± 4.34	20.42 ± 3.33	AB† AC†	0.700
Ca mg/dl	4.02 ± 1.10	4.03 ± 0.86	3.98 ± 0.76	0.992	0.520
P mg/dl	13.62 ± 2.05	16.11 ± 6.58	15.81 ± 4.08	0.600	0.121

Abbreviations: AB: indicates significant difference between group A and group B; AC: indicates significant difference between group A and group C; BC: indicates significant difference between group B and group C; *: $p < 0.01$; ‡: $p < 0.05$; †: No significant difference in post hoc comparison; Data are expressed as mean ± standard deviation.

Table 2. Correlation of Salivary Flow Rate, pH, Protein and Age

	Flow rate	pH	Protein
Age	0.132	0.089	0.479*
Flow rate		0.364*	–0.473*
pH			–0.361†

*: Correlation is significant at the < 0.01 level (2-tailed).
†: Correlation is significant at the < 0.05 level (2-tailed).

saliva pH (r = –0.361, 95% CI: –0.684 to –0.072, p < 0.01, Table 2).

DISCUSSION

We found that as the salivary flow rate increased, pH increased but protein decreased, which was in line with the literature.[4,5,18] The flow rate was negatively correlated to proteins, similar to Soderling's longitudinal study conducted on 10- to 15-year-old children.[18] However, other studies conducted in adults suggested that protein content increases proportionally to increasing flow rate.[1,5] The differences in protein content and flow rate in the literature may be due to age. Also, evidence has shown that the concentrations of proteins in the saliva of young children differ from those in adults.[16]

In our study, an age-related increase was noted in the unstimulated salivary flow rate of the preschool and elementary school groups (p < 0.05). There were no statistical differences between the junior-high school group and the other two groups. Our result was similar to that of Crossner.[19] Most studies in the literature demonstrated an age-correlated flow rate increase.[4,11,18] Evidence shows that saliva is detected from birth, flow rate increases with age and slows down after 29 years of age, due to a maturation of the central control mechanism and progressive replacement of salivary gland tissue by fat and atrophy of acinar cells.[4]

We found that the junior-high school group had a range of 0.96 m/l/min to 1.53 m/l/min. The results were in agreement with Kedjarune's 1.2 ± 0.6 ml/min to 1.0 ± 0.5 ml/min of urban children and 2.3 ± 1.2 ml/min to 2.5 ± 1.5 ml/min of rural children.[20] The results are also supported by Crossner's and Mazengo's studies.[19,21] Our results were somewhat higher than the others in the literature.[10,12,13] The higher resting flow rate may be related to collecting time as one hour post lunch is one of the peak times for flow rate during the circadian cycle.[22] Differences between the present values and those reported in the literature can also be explained by the dissimilarity in subjects, collecting methods and conditions.[12,13] In addition, saliva as a whole is very variable due to its secretion mechanism,[4] in which dentition status and developmental changes, such as body weight, height and maturation of salivary glands, may play a role.[10]

Under unstimulated conditions, we found no significant pH difference between the three age groups. The average pH values we obtained were 7.17 for the pre-school group, 7.44 for the elementary school group and 7.24 for the junior-high school group. The results were similar to some studies but slightly higher than others.[6,17,20] According to Brawley, the pH of resting saliva from subjects ranging from 3 weeks to 101 years of age does not vary significantly.[6] The general pH of resting saliva was 6.80 for 6-10 year olds (122 males and 158 females). Differences in pH can be contributed to by collecting method, dietary consumption and caries.[21]

Our protein content was positively correlated to age, with the junior-high school group having the highest value. However, our amylase content showed no inter-group difference. Significant ascending linear correlation of age to the concentration of total protein was also demonstrated by Ben-Aryeh.[16] Evidence showed that total protein correlated significantly with the size of the parotid.[13] It is plausible that a physiological growth resulted in greater parotid volume. On the other hand, since the amylase level is very variable and its activity has been recorded to reach adult values by 12 months,[11,16] this may explain the result we obtained.

For the inorganic compositions of resting saliva, we found significant differences in potassium content of the junior-high school group compared to the other two groups. Our results were similar to Ben-Aryeh for children from 6-8 years of age.[16] According to Sippell, the concentrations of potassium that change in saliva with age might be a result of hormonal development.[23] On the other hand, inter-group salivary calcium and phosphorus did not reach statistical differences among the three age groups. Salvolini studied 100 healthy subjects of both genders, aged between 10 and 80 years, and suggested that the concentrations of calcium and phosphorus were not affected by age.[24]

The major limitation of this study may be its small sample size. The data obtained in this study is preliminary and expansion of the subject is warranted for improved validation.

In conclusion, age-related increase in unstimulated salivary flow rate from elementary and preschool groups was noted. As the flow rate increases, the pH increases but the protein decreases. The information obtained may serve as reference values for the growing interest in saliva as a diagnostic tool, especially monitoring those with neurological or oral motor dysfunction.

Acknowledgements

This work was supported by a grant from the National Science Council of Taiwan (NSC92-2214-B-182A-050). The authors wish to thank Liang-Yi Yang for her assistance with the statistical analysis, and the teachers and students of Tao-Yuan County for their cooperation throughout the study.

REFERENCES

1. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent 2001;85:162-9.
2. Edgar WM, O'Mullane DM. Saliva and Oral Health. 2nd ed. London: British Dental Journal, 1996:105.
3. Edgar WM, O'Mullane DM. Saliva and Oral Health. 2nd ed. London: British Dental Journal, 1996:113.
4. Granick MS, Hanna DC. Management of salivary gland lesions. 2nd ed. Baltimore: Williams & Wilkins, 1992:21.
5. Deburgh Norman JE, McGurk M. Colors Atlas and Text of the Salivary Glands: Diseases, Disorders and Surgery. 1st ed. London: Mosby-Wolfe, 1995:47.
6. Brawley RE. Studies of the pH of normal resting saliva: variations with age and sex. J Dent Res 1935;15:55-77.
7. Kavanagh DA, Svehla G. Variation of salivary calcium, phosphate and buffering capacity in adolescents. Arch Oral Biol 1998;43:1023-7.
8. Dawes C. Circadian rhythms in human salivary flow rate and composition. J Physiol 1972;220:529-45.
9. Dawes C, Ong BY. Circadian rhythms in the flow rate and proportional contribution of parotid to whole saliva volume in man. Arch Oral Biol 1973;18:1145-53.
10. Bretz WA, do Valle EV, Jacobson JJ, Marchi F, Mendes S, Nor JE, Cancado MF, Schneider LG. Unstimulated salivary flow rates of young children. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91:541-5.
11. Dezan CC, Nicolau J, Souza DN, Walter LR. Flow rate, amylase activity, and protein and sialic acid concentrations of saliva from children aged 18, 30 and 42 months attending a baby clinic. Arch Oral Biol 2002;47:423-7.
12. Rotteveel LJ, Jongerius PH, van Limbeek J, van den Hoogen FJ. Salivation in healthy schoolchildren. Int J Pediatr Otorhinolaryngol 2004;68:767-74.
13. Ono K, Morimoto Y, Inoue H, Masuda W, Tanaka T, Inenaga K. Relationship of the unstimulated whole saliva flow rate and salivary gland size estimated by magnetic resonance image in healthy young humans. Arch Oral Biol 2006;51:345-9.
14. Navazesh M, Mulligan RA, Kipnis V, Denny PA, Denny PC. Comparison of whole saliva flow rates and mucin concentrations in healthy Caucasian young and aged adults. J Dent Res 1992;71:1275-8.
15. Fenoll-Palomares C, Munoz Montagud JV, Sanchiz V, Herreros B, Hernandez V, Minguez M, Benages A. Unstimulated salivary flow rate, pH and buffer capacity of saliva in healthy volunteers. Rev Esp Enferm Dig 2004;96:773-83.
16. Ben-Aryeh H, Fisher M, Szargel R, Laufer D. Composition of whole unstimulated saliva of healthy children: changes with age. Arch Oral Biol 1990;35:929-31.
17. Siqueira WL, de Oliveira E, Mustacchi Z, Nicolau J. Electrolyte concentrations in saliva of children aged 6-10 years with Down syndrome. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;98:76-9.
18. Soderling E, Pienihakkinen K, Alanen ML, Hietaoja M, Alanen P. Salivary flow rate, buffer effect, sodium, and amylase in adolescents: a longitudinal study. Scand J Dent Res 1993;101:98-102.
19. Crossner CG. Salivary flow rate in children and adolescents. Swed Dent J 1984;8:271-6.
20. Kedjarune U, Migasena P, Changbumrung S, Pongpaew P, Tungtrongchitr R. Flow rate and composition of whole saliva in children from rural and urban Thailand with different caries prevalence and dietary intake. Caries Res 1997;31:148-54.
21. Mazengo MC, Soderling E, Alakuijala P, Tiekso J, Tenovuo J, Simell O, Hausen H. Flow rate and composition of whole saliva in rural and urban Tanzania with special reference to diet, age, and gender. Caries Res 1994;28:468-76.
22. Edgar WM, O'Mullane DM. Saliva and Oral Health. 2nd ed. London: British Dental Journal, 1996:29.
23. Sippell WG, Dorr HG, Bidlingmaier F, Knorr D. Plasma levels of aldosterone, corticosterone, 11-deoxycorticosterone, progesterone, 17-hydroxyprogesterone, cortisol, and cortisone during infancy and childhood. Pediatr Res 1980;14:39-46.
24. Salvolini E, Mazzanti L, Martarelli D, Di Giorgio R, Fratto G, Curatola G. Changes in the composition of human unstimulated whole saliva with age. Aging (Milano) 1999;11:119-22.

台灣健康兒童口腔未受刺激之唾液流速和組成的相關性研究

吳佩璇 柯智裕 鍾佳英 陳嘉玲 黃聰龍[2] 周明嫣[1] 黃美涓 胡瀞方 李裕承

背 景： 唾液在調節口腔健康過程中是最重要的因素之一，唾液流速和組成會隨著生長發展和疾病而改變。本研究目的是在探討台灣健康兒童口腔未受刺激之唾液流速和組成的相關性。

方 法： 本研究收集 44 位 3-14 歲正常健康兒童，依年齡分爲 3 組。學齡前組 (年齡 3-5 歲)，國小組 (6-11 歲) 和國中組 (12-14 歲)。所有兒童皆在口腔未受刺激下，測試唾液流速，酸鹼值和唾液組成。研究統計使用單因子變異數分析和皮爾森相關分析。統計顯著差異定在 p 值小於 0.05。

結 果： 研究結果顯示在口腔未受刺激下，國小組的唾液流速大於學齡前組 ($p < 0.05$)。三組間的唾液酸鹼值，鈣，磷和澱粉酵素並無達到統計上顯著差異。當唾液流速增加，唾液酸鹼值隨之增加 ($r = 0.364$, $p < 0.05$)，但唾液蛋白質隨之減少 ($r = -0.473$, $p < 0.05$)。另外，研究發現唾液蛋白質與年齡成正相關 ($r = 0.479$，$p < 0.05$) 但和唾液酸鹼值成負相關 ($r = -0.361$, $p < 0.01$)。

結 論： 唾液流速從學齡前組到國小組，隨著年齡增加上升。當唾液流速增加時，唾液酸鹼值隨之增加，但唾液蛋白質隨之減少。本研究的結果可提供患有神經或口腔動作失調病童的診斷與監控之參考依據。

(長庚醫誌 2008;31:281-6)

關鍵詞： 兒童，未受刺激之唾液，組成，酸鹼值

長庚紀念醫院 台北院區 復健科，[1]牙科；長庚大學 醫學院 [2]天然藥物研究所
受文日期：民國95年7月25日；接受刊載：民國96年8月2日
通訊作者：陳嘉玲醫師，長庚紀念醫院 復健科。桃園縣333龜山鄉復興街5號。Tel.: (03)3281200轉3846; Fax: (03)3281320; E-mail: ccl1374@cgmh.org.tw