THE VARIATION OF SOME SALIVARY COMPONENTS IN CORELATION WITH SEX AND AGE AT PUBERTY

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ABSTRACT

Introduction: Saliva, a mixed serous and mucous secretion produced daily in a quantity of about 750-800 ml serves multiple and important functions. The numerous salivary components protect the oral tissues and represent biomarkers for local and systemic diseases.
Material and method: Salivary composition was studied in five age groups of 9 to 18 years old, both genders, using 100 samples of saliva, collected from children and teenagers, pupils or students in Timisoara. The sialometric determination consists of 3 parameters - pH, calcium and chlorine concentration using pH-metric and photometric methods.
Results: The variation of calcium and chlorine show greater values in males, between 11-15 years old and statistical significance for any of the age groups (e.g. 9 years old). The percentage of extreme individual values of pH is different - 1 up to 4%.
Conclusions: This correlative study can be easily extended to a higher scale of children and adolescents, especially those presenting different diseases, for monitoring the predisposition and evolution of the disease.
Key words: saliva analysis, biochemical components, different age groups, correlative study

PREMISES OF THE STUDY

Saliva is a mixed secretion produced by three pairs of major salivary glands (parotid, sublingual and submandibular) and by numerous minor glands (600-1000) widely distributed in the oral cavity and oropharynx.[1] The total volume of the saliva secreted daily by humans is about 750-800 ml [2, 3] the quoted values ranging from 600 ml/day [4] to 1-1.5 l/day. [5]

The total saliva, serous and mucous, contains minerals, electrolytes, buffers, enzymes and enzyme inhibitors, growth factors, cytokines and immunoglobulins (IgA). Once the saliva passes through the ducts and enters the oral cavity, it mixes with blood cells, microorganisms (viruses, bacteria and yeast) and microbial products, oral epithelial cells and cell products, food debris and upper airway secretions.[4]

The salivary flow is between 0.05-1.8 ml / minute when not active, possible to grow up to 2.25-5 ml/ minute at stimulation.[2]

Although saliva consists mostly of water, it plays a key physiologic role in the lubrication, repair and protection of the oral mucosa; in fact the mucin layer on the mucosal buccal cavity represents the most important non-immune defense mechanism. Saliva also contributes to lubrication. It moistens dry food and cools hot food and provides the environment in which dissolved food stimulates the taste buds. It also controls the oropharyngeal bacterial flora and represents a buffer for the oral cavity, having a high concentration of bicarbonate ions.[1]

Saliva also aids in plaque formation and, by its supersaturation with respect to tooth mineral, in the process by which dental enamel can be remineralised. Proteins that are found in the saliva, such as lactoferrin, lysozyme and histatine, can destroy or inhibit the growth of the microorganisms in the oral cavity; a case in point is histatine which has antifungal properties. Because of

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the presence of á-amylase it also participates in the digestion of starch, by breaking the 1-4 glycosidic bond.

The properties and components of saliva vary within large limits in humans, according to the secretor flow, the stimulating nature, subject, qualitative and quantitative contribution of each gland (from the total volume of saliva secreted in humans, 60% is produced by the submandibular glands, 30% by parotids, 5% by the sublingual, and roughly 4 to 7% by the accessory salivary glands - values for the stimulated secretion); [3,5] taking all these aspects into consideration it is difficult to stabilize a mean or „normal" composition of saliva.

Lately, saliva is frequently used as a biological fluid for the detection of different „biological markers", such as electrolytes, hormones, medicines and antibodies in human and veterinary medicine. Taking saliva samples is non-invasive and painless and can be easily done with various devices several times a day andit provides valuable evidence in complicated situations when blood tests are not available or are difficult to obtain.

Salivary determinations are recommended and used in physiology, sports medicine, pharmacology, pediatrics, as well as many other scientific fields. A recent pilot study published in the Journal of the Canadian Dental Association put into evidence the variations of salivary IgA in older adults from a geriatric centre related to different types of medications, mastication abilities, cortisol and total plasmatic protein concentration with the purpose of monitoring the nutritional status of the patient. The sialometric determinations were also experimentally used for establishing the moment of ovulation as well as for monitoring the plasmatic concentration of some drugs.[3]

AIMS OF THE STUDY

The aim of the present paper is the study of the salivary composition in children and adolescents at different age groups and both genders in order to get a general image of the oral cavity and to allow early diagnosis of the oral cavity, based on the cooperation between the physician and the dentist.

BIOLOGICAL MATERIAL

In the first trimester of 2003 we have collected and analyzed 100 salivary samples from children and teenagers – pupils in Elementary School No. 18 in Timisoara and first years students of the Victor Babes University of Medicine and Pharmacy - Timisoara. The subjects were divided into five age groups 9-11-13-15-18 years, each group consisting of 20 subjects - boys and girls, equally represented. None of the subjects presented an acute or chronic disease.

The samples were collected in sterile test tubes without any stimulation of the salivary secretion. The same conditions were respected for all subjects and the moment of collecting the samples was chosen to be two hours after breakfast.

METHODS

Sialometric determinations implied the following parameters: pH, calcium and chlorine concentration:[6]

• pH determination with the pH-meter TITRO – LINE EASY - SCHOTT

• ionic determination was realized through the photometric method using mercuric thiocyanide and iron nitrate for chlorine and glyoxal-bis (2-hydroxyalanil) for calcium.

Data processing consisted of:

• calculation of mean values (m), standard deviation (s) and standard error of the mean values (sm) for all parameters

• statistical analysis using one-tailed Student-t test for the differences between male and female subjects

RESULTS AND DISCUSSIONS

The salivary pH variation with age and gender

The mean value of pH of the non-stimulated total saliva is 6.0 (5.75 - 6.15) and depends mostly on the bicarbonate content which comes partially from plasma and partially from activity of the salivary glands which contain carbonic anhydrase.[2] pH can be influenced by the salivary flow and diet. The mean pH value determined in female subjects varied between 6.15 (11 years old) and 6.58 (15 years old) and in male subjects between 6.36 (at 11 and 18 years old) and 6.76 (15 years old).

The variation curve of pH with the age indicates higher values in male subjects compared to the female subjects except the group of 9 years old; the differences between the two genders do not have a statistical significance ($p > 0.05$).

The literature does not describe different salivary composition and properties between the two genders, but according to age a more alkaline pH has been shown in children (pH > 7)[5] and after stimulation as well.

The individual minimal values have been registered in 1% of the subjects - at 11 years old (pH = 5.5) and 18 years old (pH = 5.7) for females and at 9 and 18 years old (pH = 6) for males. Maximal values were registered in 4% of the female subjects (pH = 7.1 at 13 years old) and in 2% of the male subjects (pH = 7.4 at 15 years old).

Table 1. Mean values (m), standard deviations (s), and standard error of the mean values (sm) for all studied parameters

Age (years)	Gender	Values	pH	Chlorine (mmol/l)	Calcium (mmol/l)
	F	**m**	**6,56**	**45,156**	**0,596**
		s	0,134164	10,15413	0,19139
		sm	0,06	4,541064	0,085592
9	M	**m**	**6,45**	**50,93**	**0,975**
		s	0,412311	11,56185	0,208087
		sm	0,206155	5,780924	0,104043
	F	**m**	**6,15**	**43,26**	**1,2675**
		s	0,750555	4,084434	0,195683
		sm	0,375278	2,042217	0,097841
11	M	**m**	**6,36**	**45,852**	**1,142**
		s	0,497996	11,47042	0,414451
		sm	0,222711	5,129727	0,185348
	F	**m**	**6,42**	**45,302**	**1,154**
		s	0,571839	6,696101	0,291685
		sm	0,255734	2,994587	0,130445
13	M	**m**	**6,433333**	**48,49667**	**0,858333**
		s	0,361478	7,516027	0,395344
		sm	0,147573	3,068405	0,161398
	F	**m**	**6,58**	**48,212**	**0,856**
		s	0,130384	2,854044	0,213963
		sm	0,05831	1,276368	0,095687
15	M	**m**	**6,766667**	**44,11667**	**0,803333**
		s	0,417931	6,509773	0,398229
		sm	0,17062	2,657604	0,162576
	F	**m**	**6,3**	**44,03**	**1,232**
		s	0,514782	7,190643	0,458607
		sm	0,230217	3,215753	0,205095
18	M	**m**	**6,36**	**45,458**	**1,476**
		s	0,230217	3,282723	0,307132
		sm	0,102956	1,468078	0,137354

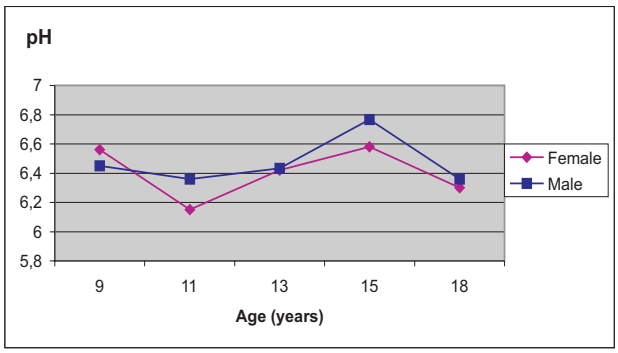

Figure 1. Salivary pH variation with age and gender

In the general population, pH values lower than 6.2 or superior to 7.4 have been registered only in 2%.[5]

An acid pH lower than 5.5 (considered to be critical) favorises the solubility of calcium salts and therefore the process of caries formation, while an alkaline pH favourises the formation of plaque and salivary lithiasis.

Variation of the salivary ions with age and gender

The anorganic components of the saliva represent approximately 0.2-0.3% of the total constituents of saliva which means approximately 1/3 of the total dry substance. Although the salivary ions are the same with the plasmatic ones, the relative concentration of Na^+, K^+, Mg^{2+}, Ca^{2+} and Cl^- and also SO_4^{2+}, $H_2PO_4^-$ and HPO_4^- indicates that saliva is not just a simple plasmatic ultrafiltrate. A part of those ions, including Ca^{2+} and Cl^- are found in saliva generally in lower concentrations than in plasma while phosphate and especially potassium are presenting higher values than in plasma.[2]

Some authors describe the salivary concentration of calcium as being comparable with the plasmatic one, recommending the determination of this ion in saliva instead of serum.

The salivary calcium is found as non-permeable and non-dyalitic. Approximately 3% of the total quantity of calcium is bound to mucin according to the salivary pH. At pH = 6 a mol of mucin binds 10.8 mol of calcium and at pH = 9 the number rises to 35.4 mol.[7]

The calcium suprasaturation is maintained by the specific molecules in parotid and submadibular saliva which are bound to the calcium phosphate surface preventing the spontaneous precipitation and growth of the hydroxyapatite crystals.

Those molecules include anionic proteins rich in prolin, as well as staterine (B protein) - a polypeptide rich in phosphorous which, besides the inhibition of the precipitation has a transporting role of calcium and

phosphates from the salivary secretion and the maintenance of the enamel surface integrity while the lowering of pH. It inhibits also the calcification inside salivary glands and ducts.[8, 9]

The mean values of the salivary calcium differ in literature (Tab. 2):

Table 2. Mean values of salivary and plasmatic calcium in literature

Salivary concentration	Plasmatic concentration	Source
1-2 mmol / l	2,5 mmol / l	Cole & Eastoe, 1988
0,6 - 8 mmol / l sau 1,5 mEq / l sau 5,8 mg%		Ionescu, 1998
2,5 - 5,5 mEq / l	5 mEq / l	Aurora Popescu, 1992

The calcium ion is the most sensitively regulated among the plasmatic electrolytes so that it remains relatively constant even if its quantity increases in diet. Calcium plays, along with fluoride, glucides and D vitamin a direct role in the relationship between tooth-saliva-bacteria and in the incidence of dental diseases.[2,13]

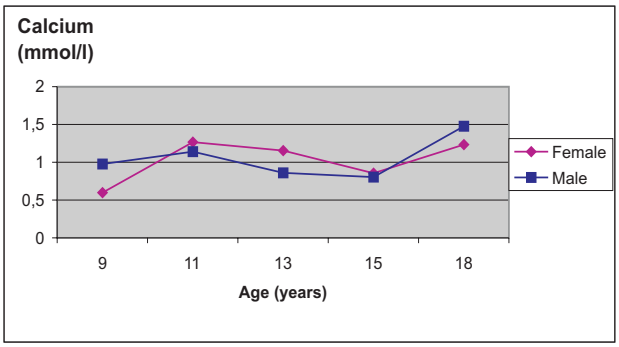

Figure 2. Salivary calcium variation with age and gender

In our sample the mean values of salivary calcium in female subjects was between 0.596 mmol/l (9 years old) and 1.26 mmol/l (11 years old), this value being close to the one at 18 years old and for boys between 0.80 mmol/l (15 years old) and 1.47 mmol/l (18 years old). In male subjects the variation curve presents an ascending aspect and the mean values are lower than in female subjects between 11 and 15 years old. In girls the variation curve is having also an ascending aspect, except for the 15 year old group.

The Student–t test shows statistical significant differences in-between the two genders only at 9 years old ($p < 0.01$).

Variation of chlorine is among 17 and 29 mmmol/l, [1] 5-70 mEq/l [4] and 50 mg% (nonstimulated saliva) and 100 mg% (stimulated saliva)[8,14,15] and it does not follow the variation of plasmatic chlorine. The salivary chlorine increases after parasympathetic stimulation in parotid saliva and it decreases after food stimulation.

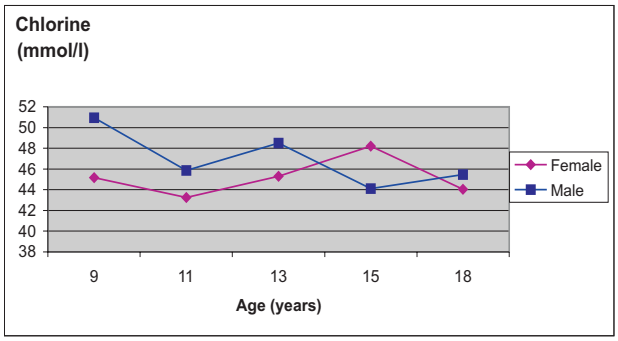

Figure 3. Salivary chlorine variation with age and gender

The salivary chlorine activates the salivary amylase and along with sodium are in lower concentration compared to plasma.

In our sample the minimum value was registered at 11 years old for female subjects (43.26 mmol/l) and the maximum value at 15 years old (48.21 mmol/l). In male subjects the maximum value is 50.93 mmol/l (9 years old) and minimum value 44.11 mmol/l (15 years old).

Generally, for chlorine, the values are higher in male than in female subjects, situation which is opposite for calcium.

CONCLUSIONS

1. The mean values of pH varied between the limits described in literature but the percentage of extreme individual values is different – 1 up to 4 %.

2. The salivary calcium displayed mean values slightly higher in male than in female although in female it is higher between 11 and 15 years old. Student-t test revealed statistical significance between the two genders just in the 9 years old group.

3. The salivary chlorine had higher mean values in boys than in girls except for the 15 years old group. The Student-t test doesn't indicate statistical significance between the two sexes ($p > 0.05$).

4. The mean values of all studied parameters varied within normal values (quoted in literature). This type of correlative study can be easily extended to a higher scale of children and adolescents, especially those presenting different diseases, for monitoring the predisposition and evolution of the disease.

5. Although the literature[10,11] data indicates a low frequency of oral cavity diseases in childhood, any symptom would indicate a pathological situation which must be evaluated both by the physician and the dentist.

6. Due to the multiple factors which influence both salivary flow and salivary components, it is difficult to establish a mean composition, „normal", valid at any age. Following the variation of the salivary parameters according with the age and gender can help in the early diagnosis of oral cavity diseases. [12, 16]

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