Concentrations of selected elements in saliva among a group of adolescent girls in relation to severity of caries and selected salivary parameters

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ABSTRACT

Background: Saliva is one of the most important etiological host factors in relation to dental caries. It affects the carious process by its organic and inorganic constituents; in addition to its physiological functions as (flow rate, pH and buffer capacity). The aims of this study were to determine the concentrations of major elements (calcium and phosphorus) and trace elements (ferrous iron, nickel, chromium and aluminum) in saliva among a group of adolescent girls, and to explore the relation of these elements, flow rate and pH with dental caries.

Material & Methods: The study group consisted of 25 girls with an age of 13-15 years old. Dental caries was diagnosed by both clinical and radiographical examinations following the criteria of D_{1-4}MFS index. Stimulated saliva was collected from patients between 9-11 Am under standarized conditions, and chemically analyzed to determine the concentration of calcium, nickel, chromium and aluminum by Atomic Absorption Spectrophotometer, while salivary phosphorus and ferrous iron were determined by using colorimetric method. The average salivary flow rate was measured from total volume, and salivary pH was determined using digital pH meter. All data were analyzed using SPSS version 19.

Results: All elements measured in saliva in addition to P/Ca ratio recorded statistically non significant correlation with DMFS, except ferrous Fe ions which showed statistically significant correlation (r= 0.34, P=0.05). Salivary flow rate and pH correlated weakly and statistically not significant with DMFS

There were weak and statistically not significant correlations between all elements measured in saliva and salivary flow rate and pH.

Conclusions: It had been found that Fe, Ni, Al and Cr ions present in very small amounts in saliva in comparison to Ca and P ions. The presence of these elements in saliva may indicate their presence in food, water and air.

Key words: Trace elements, salivary pH and flow rate, caries severity. (J Bagh Coll Dentistry 2013; 25(1):171-175).

INTRODUCTION

Saliva is one of the most important host factors that play a role in the caries process through its organic and inorganic constituents, besides the physiological functions. The continuous flow of saliva through the mouth bathes the dentition with remineralizing ions and removes cariogenic challenges [(1-3)]. Therefore saliva plays an important role in the equilibrium between the demineralization and the remineralization of enamel [(4)]. Trace elements in saliva in correlation to dental caries were investigated by different observational studies including Iraqi ones and a great controversy was observed [(5-8)]. In order to increase the knowledge about the role of inorganic elements in relation to dental caries severity, this study was designed.

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MATERIALS AND METHODS

The total number of patients was 25 girls with an age rang of 13-15 years, recorded according to the last birthday [(9)]. Examinations were carried out in the specialized dental center in Al-Sader City and the specialized center for Prosthodontic and Orthodontic treatment in Al-Qaira in Baghdad province under standarized conditions [(9)]. Dental caries was diagnosed by both clinical and radiographical examinations. The clinical examination of teeth surfaces was done by using dental mirror and sharp dental explorer. Assessment and recording of caries experience was done by the application of (D_{1-4} MFS index for permanent teeth) [(10)].

Prior to clinical examination, stimulated saliva was collected from patients between 9-11 AM. Each patient was asked not to eat or drink (except water) 1 hour before collection, saliva, if possible, should be collected at the same time of day from the same subject, the patient should not smoke or undergo heavy physical stress before collection, a pre-sampling period (1min) is recommended with a fixed collection time, the patient should sit in relaxed position, acute illnesses or chronic diseases as well as medication should be considered, samples containing blood should be discarded if chemical analysis of saliva is planned [11]. Each patient was asked to chew apiece of Arabic gum (0.35- 0.4 gm) for one minute then to remove all saliva by expectoration. Chewing was continued for five minutes, with the same piece of gum and saliva collected in sterile screw capped bottle. After collection and disappearance of salivary foam, the pH of saliva was measured using a digital pH meter. Salivary volume was estimated and the rate of secretion was expressed as milliliter per minute (ml/ min). Each salivary sample was then centrifuged by centrifugater at 3000 r.p.m. (revolution per minute) for 10 minutes. Salivary supernatant was stored at (-20ºC) in polyethylene tubes for subsequent chemical analysis.

Chemical analysis was carried out at Poisoning Consultation Center, Medical city. Calcium, aluminum, nickel and chromium ions were determined using Air-acetylene Atomic Absorption Spectrophotometer (AAS), while phosphorus and iron were determined color-metrically by using chemical kits. SPSS version 19 (Statistical Package for Social Sciences) was used for statistical analysis. Descriptive measurement (mean and standard deviation) were used to describe variables. The statistical significance, directions and strength of linear correlation between the concentration of element in each sample and values of D_{1-4}MFS index was measured by Person's correlation coefficient. Multiple linear regressions between dependant variable (dental caries) and independent variable (concentration of elements) were applied. P value equal to or less than (0.05) level of significance was considered to be statistically significant. The confidence limit was accepted at 95%.

RESULTS

Clinical and radiographical examinations showed that all subjects were affected by dental caries. Decayed, missed and filled teeth surfaces of girls by fractions of D_{1-4}MFS index were represented by their means and standard deviation (SD) in Table 1. The decayed surfaces (DS) contributed the major parts of this index followed by filled surfaces (FS) then missed surfaces because of caries (MS). Grade (2) of lesion severity was the highest one, while the frank cavitation; grade (4) was the lowest one.

The rang of flow rate was recorded to be 0.6 to 1.8 ml/ min with (1.07 ml/min ± 0.35) mean, while the range of salivary pH was recorded to be 5.3 to 7.5 with (6.54 ± 0.6) mean.

Table 2 illustrates the concentration of elements in saliva. Phosphorus ions were the highest followed by Ca ions then ferrous Fe, Al, Ni and finally Cr ions.

Table 3 illustrates the correlation coefficient between elements in saliva with salivary flow rates and pH. It had been found that all elements measured in saliva and P/Ca ratio correlated weakly with salivary flow rate and pH, where all of these correlations were statistically not significant.

Table 4 illustrates the correlation coefficient between salivary (elements and parameters) and caries-experience. A negative strong and statistically highly significant correlation was recorded between P ions and D_1; on the other hand a positive weak and statistically significant correlation was recorded between ferrous Fe ions and DMFS. While other elements measured in saliva in addition to P/Ca ratio showed weak and statistically not significant correlations with caries-experience where some of them were positive while others were negative. Regarding the correlations recorded by salivary parameters with caries-experience. It had been found that salivary flow rate and pH recorded negative but statistically not significant correlations with DS and DMFS. While a negative and statistically significant correlation was recorded between salivary flow rate and D_3. Other correlations recorded by these two parameters and caries-experience were weak and statistically not significant.

The results of MLR for the DMFS (dependant variable) explained by elements measured in saliva (independent variable) were illustrated in Table 5. A complete correlation coefficient of 0.516 was recorded between DMFS and all factors entered. The R^2 value of 0.266 was recorded indicated that 26.6% of changes occurred in DMFS were explained by the inorganic composition of saliva. The highest beta-coefficients was recorded for P ions, while the lowest one was recorded for P/Ca ratio. Beta-coefficients of all elements measured in saliva were recorded to be statistically not significant.

Table 6 illustrates the results of MLR for DMFS (dependent variable), explained by salivary flow rate and pH (independent variable). A complete correlation coefficient of 0.323 was recorded between DMFS and (salivary flow rate and pH). R^2 value of 0.104 was recorded indicated that 10.4 % of changes occurred in DMFS were explained by salivary flow rate and pH. Beta-coefficient recorded for salivary pH was higher than that recorded for salivary flow rate; however these two coefficients were statistically not significant.

DISCUSSION

The study group in this research involved 25 girls with an age range 13-15 years old. Boys were not involved in this study due to the variation in size of salivary glands that results in variation in composition and flow rate of saliva in addition to variation in adolescent time, hormonal variation and differences in life style [12].

Stimulated saliva was collected rather than unstimulated saliva, in order to allow comparison with other Iraqi studies that were mostly performed on stimulated saliva. In addition to that stimulated saliva is easier and more standardized to collect.

In this study calcium and inorganic phosphate were determine in saliva. Results showed that P ions concentration (84.62ppm) were higher than Ca ions (80.60ppm). This result could be explained by depending on stimulated saliva in this study. In the stimulated saliva there will be an increase in the concentration of inorganic phosphate in comparison to calcium and the level of this elements is time- dependent, as it increase with the increase in the duration of stimulation, while Ca decrease when going from unstimulated to stimulated saliva [2, 3, 13]. In addition to that there is an increase in the proportion of parotid saliva in stimulated saliva that characterized by reduction in Ca ions and increase in P ions [14, 15]. Therefore P/Ca ratio in saliva could determine the differences between these two ions according to the duration of stimulation.

The concentration of Ca and P ions recorded here were higher than that recorded by other Iraqi studies [7, 16-18]. The variation in the sampling procedure a well as techniques of analysis in addition to difference in ages of the study groups may explain the variation in these Iraqi studies and others.

Other elements namely ferrous iron, nickel, aluminum and chromium were detected to be percent in saliva with the concentrations as seen in Table 2. These elements present in our environment; as they present in foods as (meats, potatoes, cheeses, whole-grain breads and cereals, fresh fruits and vegetables, chicken, eggs, milk, nuts, dried beans and peas). These elements could also be found in water that used for drinking or cooking foods and in air as pollutant as for Cr VI that presents in air due to erosion of chromium-containing rocks and nickel that presents in cigarettes that may inhale by those group as passive smokers [19, 20], so they enters the blood stream via digestive system, lungs or even by coming in contact with skin. The presence of these elements in the blood serum, allowed them to be introduced in whole saliva via gingival crevicular fluid or by incorporating the pure saliva by reaching salivary glands through serum or by both [21, 22]. This could explain the presence of these elements in saliva.

Regarding the relations with dental caries, Ca and P ions in this study correlated negatively with DMFS. Although they were not significant, these correlations could indicate the important role of saliva in the protection of tooth surface against caries development by maintaining supersaturation of Ca and P ions in saliva. Other elements studied in saliva except Fe showed negative correlations with caries-experience that was statistically not significant. These elements showed the same correlations with D_1 and D_4. These results indicating that when these elements increased in saliva caries severity decreased, so they may act as cariostatic elements in saliva. These results were confusing, since saliva is the main source of these elements in the outer enamel surface, and these elements were recorded by other studies to act as cariogenic elements [23, 24]. It had been found that the concentration of these elements in saliva changed continuously since it depend on their presence in systemic environment that affected by type of food, water, air and even drugs as for iron supplements [25]. While caries process is longitudinal process and depend on interaction of a large number of factors with time [26].

Salivary flow rate and pH considered to be an indicator of caries susceptibility, as the reduction in salivary flow rate can eventually results in reduction of its protective constituents and functions including salivary buffer system, this may increase susceptibility for dental caries [14, 15]. There is an inverse association between caries-experience and these two variables; such negative correlation was also recorded in the present study.

The impact of elements measured in saliva on dental caries seems to be much more in comparison to that of salivary parameters (flow rate and pH). As results recorded a value of R^2 equal to 0.266 for elements in saliva compared to only 0.104 for salivary parameters indicating that 26.6% of changes occurred in caries-experience were explained by inorganic composition of saliva, while salivary flow rate and pH explain only 10.4% of these changes. This could explain the important role of inorganic composition of saliva in the initiation of dental caries by incorporation of its inorganic elements in outer enamel surface during the demineralization and remineralization processes.

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Table 1: Caries – Experience of Permanent Teeth (D_{1-4}MFS) among Patients.

Fractions	Mean ± SD
DS	4.44 ± 2.48
MS	1.60 ± 2.78
FS	2.60 ± 2.60
DMFS	8.64 ± 4.06
D_1	0.84 ± 0.68
D_2	1.76 ± 0.87
D_3	1.20 ± 0.95
D_4	0.64 ± 1.11

Table 2: Concentration of Elements in Saliva (Means ± SD)

Elements	Mean ± SD (ppm)
Ca	80.60 ± 22.97
P	84.62 ± 01.72
P / Ca	1.18 ± 00.51
Fe	0.69 ± 00.28
Ni	0.07 ± 00.01
Al	0.24 ± 00.10
Cr	0.06 ± 00.02

Table 3: Correlation Coefficient between Elements Measured in Saliva and Salivary (Flow Rate and pH).

Elements	pH		Flow rate	
Ca	R	0.00	R	0.21
	P	1.00	P	0.29
P	R	0.07	R	0.28
	P	0.16	P	0.16
P/Ca	R	0.06	r	-0.18
	P	0.74	P	0.36
Fe	R	- 0.21	r	-0.31
	P	0.30	P	0.12
Al	R	0.17	r	- 0.07
	P	0.41	P	0.71
Ni	R	0.11	r	- 0.02
	P	0.58	P	0.91
Cr	R	0.16	r	0.00
	P	0.41	P	0.97

Table 4: Correlation Coefficients between Salivary (Elements and Parameters) and Caries-Experience

Elements		D_1		D_2		D_3		D_4		DS		FS		DMFS	
		r	P	r	P	r	P	r	P	r	P	r	P	r	P
Ca		0.03	0.88	0.25	0.22	0.20	0.32	- 0.06	0.74	0.14	0.48	0.16	0.42	- 0.04	0.82
P		- 0.55**	0.004	0.12	0.56	- 0.19	0.36	0.09	0.65	- 0.14	0.50	- 0.10	0.62	- 0.14	0.48
P/Ca		- 0.20	0.32	- 0.18	0.38	- 0.28	0.17	0.08	0.67	- 0.20	0.31	- 0.16	0.43	- 0.11	0.58
Fe		0.26	0.20	- 0.01	0.61	0.11	0.57	0.00	0.98	0.08	0.69	0.11	0.57	0.34*	0.05
Al		- 0.05	0.81	- 0.09	0.66	0.11	0.58	- 0.04	0.84	- 0.02	0.92	- 0.27	0.17	- 0.09	0.63
Ni		- 0.35	0.08	0.00	0.97	0.00	0.98	- 0.05	0.80	- 0.12	0.56	- 0.22	0.27	- 0.03	0.87
Cr		- 0.19	0.35	- 0.12	0.55	- 0.35	0.08	- 0.12	0.54	- 0.28	0.16	- 0.01	0.96	- 0.28	0.16
Salivary parameters	F.R	- 0.28	0.16	- 0.00	0.97	- 0.40*	0.04	- 0.18	0.36	- 0.33	0.09	0.28	0.16	- 0.26	0.19
	pH	- 0.19	0.36	0.05	0.77	- 0.32	0.10	- 0.21	0.30	- 0.21	0.29	0.02	0.89	- 0.22	0.29

Table 5: Multiple Linear Regressions (MLR) Between Elements Measured in Saliva and DMFS

Elements	B (Slope)	Std. Error	Beta	t	P- Value
Ca	- 0.111	0.119	- 0.627	- 0.930	0.365
P	0.048	0.099	0.139	0.485	0.634
P/Ca	- 5.771	5.867	- 0.727	- 0.984	0.339
Fe	4.764	3.107	0.333	1.533	0.144
Al	- 3.412	3.094	- 0.252	- 1.103	0.285
Ni	- 0.313	0.856	- 0.083	- 0.366	0.719
Cr	- 7.553	43.674	- 0.318	- 1.318	0.205

R= 0.323, R^2= 0.104

Table 6: Multiple Linear Regressions (MLR) between Salivary (Flow Rate and pH) and DMFS

Salivary variables	B (slop)	Std. Error	Beta	t	Sig.
Flow rate	- 2.695	2.302	- 0.239	-1.171	0.254
pH	- 1.235	1.357	- 0.186	- 0.910	0.373

R= 0.516
R^2= 0.266