

Salivary antioxidants and physicochemical characteristics related to dental caries experience among a group of old adults

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ABSTRACT

Background: Old adults are regarded as an important target group with special oral health needs. Salivary gland structure and saliva composition particularly the antioxidants are subjected to significant changes with advancing age. The aims of this study were to assess salivary antioxidants and lipid peroxidation biomarker (malondialdehyde) levels in addition to salivary physicochemical characteristics and their effect on dental caries among a group of old adults in comparison with middle-aged.

Materials and methods: The study group consisted of all old adults (35 subjects) aged 55-65 years in comparison with all middle-aged (35 subjects) aged 30-40 years at the Textile factory in Mosul city who fitted the criteria of the study. Dental caries was recorded through the application of D₁₋₄ MFS index. Plaque and calculus indices were used for recording oral cleanliness. Unstimulated salivary samples were collected and salivary flow rate and pH were determined. Salivary samples then were chemically analyzed for the detection of salivary antioxidants (total protein, albumin, vitamin E, vitamin C and uric acid) and lipid peroxidation biomarker (malondialdehyde) in addition to salivary constituents as urea, calcium, phosphorous and magnesium.

Results: Salivary antioxidants level (total protein, albumin, vitamin E, and vitamin C) was lower among old adults compared to middle-aged ones with significant difference for vitamin C only. Malondialdehyde was slightly higher among old adults with no significant difference. Statistically no significant difference could be found regarding salivary flow rate and pH between the two age groups. Also salivary constituents (urea, calcium, phosphorous and magnesium) showed no significant difference between the two age groups. Caries experience (DMFS) was highly significantly higher among old adults (28.71±9.15) compared with middle-aged (20.68±8.53). Multiple linear regression analysis revealed inverse highly significant β coefficient for vitamin E and salivary flow rate on DS among old adults.

Conclusion: Dental caries revealed higher severity among old adults. Salivary antioxidants and physicochemical characteristics were found to affect dental caries experience among old adults.

Key words: Salivary antioxidants, dental caries, old adults. (J Bagh Coll Dentistry 2009; 21(4):108-112)

INTRODUCTION

Aging is a normal part of human development that is associated with significant changes in the function of most organs and tissues ⁽¹⁾. A number of age-related changes affect the oral structure including dental hard and soft tissues and are of clinical relevance. Descriptive morphological studies showed age-related changes in salivary parenchymal tissues with reduction in acinar cell mass and an increase in fibrous and fatty tissues ⁽²⁾. Dental caries is one of the most prevalent oral health problems ⁽³⁾. The disease is a multifactorial in etiology which depends on the interaction of three main group factors including host, microbial and substrate factors ⁽⁴⁾. The correlation between salivary physicochemical characteristics and dental caries was studied extensively but the results were controversial ⁽⁵⁻⁸⁾. Reactive oxygen species were found to play a role in dental caries process ⁽⁹⁾. Salivary antioxidant system reduces the susceptibility to dental caries because of its free radical scavenging action and other mechanisms ⁽¹⁰⁾.

As far as it is known, there are no previous Iraqi studies concerned with the relation between reactive oxygen species, salivary antioxidants and dental caries among old adults; therefore, it was decided to conduct this study.

MATERIALS AND METHODS

The studied sample is consisted of all old adults (35 subjects) aged 55-65 years and all middle-aged (35 subjects) aged 30-40 years who work at Textile factory in Mosul city. They should be non-smoker, with no medical history (depending on the medical report supplied by the medical unit at the factory) that compromises salivary secretory mechanism, shouldn't take any medications with xerogenic effect or any nutritional supplementation, and shouldn't wear any fixed or removable dental prostheses. The collection of unstimulated salivary samples was performed according to the instructions cited by Tenovuo and Lagerlöf ⁽¹¹⁾. Salivary pH immediately was measured using an electronic pH meter and flow rate of saliva was expressed as milliliter per minute (ml/min). Then salivary samples were taken to the laboratory for biochemical analysis at the College of Veterinary and College of Dentistry, University of Mosul.

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Plaque index (PII) by Silness and Løe⁽¹²⁾ was used to assess the plaque accumulation. While dental calculus was assessed according to the Calculus Index system (Call) by Ramfjord⁽¹³⁾. Gingival inflammation was evaluated by using the gingival index (GI)⁽¹⁴⁾. Caries experience was recorded according to Decayed, Missing and Filled (D₁₋₄ MFS) Index described by Mühlemman⁽¹⁵⁾. Salivary antioxidants were determined by photometric methods. Some were measured by manual methods as in case of total protein using Biuret method⁽¹⁶⁾, vitamin E depending on Emmerie-Engel reaction⁽¹⁷⁾, vitamin C by using 2, 4-dinitrophenyl hydrazine (DNPH) method⁽¹⁸⁾ and salivary MDA using the method of Beng and Aust⁽¹⁹⁾. Others were measured by using ready kits as in case of albumin and uric acid (BioMérieux sa, France). Concerning salivary urea, calcium, phosphorous and magnesium, they were determined colorimetrically using ready kits supplied by (BioMérieux sa, France) except for magnesium that supplied by (Human, Germany). Data analysis was conducted through the application of the SPSS (version 12). The Student's t-test and Multiple Linear Regression test were applied. The confidence limit was accepted at 95% ($P < 0.05$).

RESULTS

The only significant difference was found for vitamin C that was significantly lower among old adults than middle-aged ($t=2.37$, $P < 0.05$, $df=68$). Other antioxidants (total protein, albumin, and vitamin E) showed lower mean values among old adults though with no significant difference. Malondialdehyde was slightly higher among old adults with no significant difference ($P > 0.05$) (Table 1). Table 2 reveals statistically no significant difference regarding salivary flow rate and pH between the two age groups ($P > 0.05$). Also salivary constituents (urea, calcium, phosphorous and magnesium) showed no significant difference between the two age groups ($P > 0.05$).

Caries experience (mean and standard deviation) is shown in Table 3. Results revealed that caries experience represented by DMFS was highly significantly higher among old adults compared to middle-aged ($t=-3.80$, $P < 0.01$, $df=68$). Decayed surface value was highly significantly higher among old adults ($t=-3.32$, $P < 0.01$, $df=68$). Missing surface value was higher among old adults than middle-aged with no significant difference. D₂ and D₄ values were highly significantly and significantly higher among old adults than middle-aged respectively ($t=-3.21$, $P < 0.01$, $df=68$; $t=-2.63$, $P < 0.05$, $df=68$,

respectively). Table 4 demonstrates that mean PII was highly significantly higher among old adults ($t=-3.25$, $P < 0.01$, $df=68$). Result of multiple linear regression of decayed fraction (DS) of dental caries (dependent variable) explained by salivary and oral health variables (independent variables) are shown in Table 5. Among old adults, complete correlation coefficient (r) between them was 0.96 with R^2 value of 91%. Statistically highly significant beta coefficients were recorded for vitamin E, uric acid, MDA, flow rate, pH, calcium, phosphorous, and PII in addition significant beta coefficient was recorded for magnesium, but negative trends were recorded only in case of vitamin E, MDA, salivary flow rate and phosphorous. Among middle-aged adults a complete correlation coefficient between them was 0.74 with R^2 value of 54%. For all entered factors beta coefficient slopes were not statistically significant.

DISCUSSION

Unstimulated whole saliva was collected in the current study to provide a more accurate account of oral environment and saliva antioxidant composition for analysis⁽²⁰⁾. A reduction in the protective antioxidant mechanism was reported among old adults in the present study as vitamin C level decreased significantly with age also other antioxidants (total protein, albumin, and vitamin E) decreased among old adults though with no significant difference. This could be attributed to lower intake of antioxidant nutrients especially fresh fruits, vegetables and meat among old adults probably because of higher number of missing teeth among them and so reduced masticatory performance⁽²¹⁾. Also this is partially confirmed in the current study by higher missing surface value among old adults compared with middle-aged but with no significant difference. Another explanation is the elevated free radical generation with aging so salivary antioxidants would be exhausted in reaction with the elevated free radicals⁽²²⁾.

In the current study the difference between the two age groups in unstimulated salivary flow rate didn't reach any statistical significance probably due to the fact that all subjects in the present investigation were healthy unmedicated subjects since salivary flow rate is relatively unaffected by age-related changes in the structure of salivary glands in the absence of additional challenges like systemic diseases and medications probably due to the significant functional reserves within salivary glands⁽²³⁾.

With increasing age dental caries becomes a substantial oral health problem⁽³⁾; this indicates

the cumulative nature of dental caries with advancing age⁽²⁴⁾. Similarly in the present study old adults showed highly significantly higher dental caries intensity than middle-aged adults. Higher caries experience among old adults probably related to:

1. Poor oral hygiene among old adults as they reported highly significantly higher plaque accumulations than middle-aged. This is confirmed by the positive highly significant β coefficient for PII on DS among old adults.

2. Lower antioxidant protection as indicated by lower salivary antioxidants (i.e. total protein, albumin, vitamin E, and vitamin C) among old adults. This is further supported by the inverse highly significant β coefficient for vitamin E on DS. This is probably because these antioxidants inhibit oxidative carbohydrate metabolism involved in dental caries production at the local level⁽¹⁰⁾. In addition vitamin E might affect dental caries occurrence through its immunenhancing effect⁽²⁵⁾.

Table 1: Salivary antioxidants and lipid peroxidation biomarker (malondialdehyde) (Mean±S.D.) among old adults and middle-aged.

Variable (mg/dl)	Old adults (55-65 years)			Middle-aged (30-40 years)			Statistical difference		
	No.	Mean	±SD	No.	Mean	±SD	t-test	df	P-value
Total protein	35	380.89	291.10	35	541.54	419.12	1.86	68	0.07
Albumin	35	16.53	1.94	35	17.16	1.68	1.46	68	0.15
Vitamin E	35	0.18	0.13	35	0.22	0.17	1.01	68	0.31
Vitamin C	35	0.02	0.12	35	0.64	1.55	2.37	68	0.02*
Uric acid	35	6.47	1.35	35	5.73	1.12	-2.49	68	0.2
MDA (µmol/L)	35	0.16	0.15	35	0.15	0.18	-0.25	68	0.80

*Significant (P<0.05)

Table 2: Salivary physicochemical characteristics (Mean±S.D.) among old adults and middle-aged.

Variable (mg/dl)	Old adults (55-65 years)			Middle-aged (30-40 years)			Statistical difference		
	No.	Mean	±SD	No.	Mean	±SD	t-test	df	P-value
Flow rate (ml/min)	35	0.38	0.24	35	0.46	0.21	1.39	68	0.17
PH	35	7.28	0.43	35	7.17	0.50	-1.00	68	0.32
Urea	35	54.43	15.83	35	50.69	15.81	-0.99	68	0.33
Calcium	35	9.32	2.13	35	9.33	1.57	0.007	68	0.99
Phosphorous	35	12.07	4.48	35	10.95	3.11	-1.21	68	0.23
Magnesium	35	0.53	0.43	35	0.45	0.33	-0.84	68	0.40

Table 3: Caries experience (Mean±S.D.) among old adults and middle-aged

Variable	Old adults (55-65 years)			Middle-aged (30-40 years)			Statistical difference		
	No.	Mean	±SD	No.	Mean	±SD	t-test	df	P-value
D ₁	35	0.91	0.98	35	0.80	1.02	-0.48	68	0.63
D ₂	35	7.00	3.41	35	4.46	3.21	-3.21	68	0.00**
D ₃	35	1.68	1.74	35	2.31	2.04	1.38	68	0.17
D ₄	35	2.43	3.32	35	0.77	1.70	-2.63	68	0.01*
DS	35	12.02	4.89	35	8.34	4.38	-3.32	68	0.00**
MS	35	13.91	10.46	35	9.60	8.76	-1.87	68	0.07
FS	35	2.74	4.98	35	2.74	5.84	0.00	68	1.00
DMFS	35	28.67	9.15	35	20.68	8.53	-3.80	68	0.00**

*Significant (P<0.05)

** Highly significant (P<0.01)

Table 4: Plaque and calculus indices (Mean±S.D.) among old adults and middle-aged.

Variable	Old adults (55-65 years)			Middle-aged (30-40 years)			Statistical difference		
	No.	Mean	±SD	No.	Mean	±SD	t-test	df	P-value
PII	35	0.87	0.35	35	0.61	0.33	-3.25	68	0.00**
CaII	35	0.66	0.42	35	0.56	0.42	-0.95	68	0.35

** Highly Significant (P<0.01)

Table 5: Multiple linear regression of the DS explained by salivary and oral health variables among old adults and middle-aged.

Parameter	Old adults 55-65 years				Middle-aged (30-40 years)			
	β (slope)	S.E.	t	P-value	β (slope)	S.E.	t	P-value
Total protein	0.003	0.002	1.33	0.20	0.001	0.002	0.29	0.78
Albumin	-0.17	0.24	-0.70	0.49	0.39	0.45	0.88	0.39
Vitamin E	-32.21	5.20	-6.20	0.00**	5.71	5.24	1.09	0.29
Vitamin C	6.07	3.25	1.87	0.08	0.44	0.66	0.67	0.51
Uric acid	2.59	0.62	4.16	0.00**	-0.33	0.81	-0.41	0.69
MDA	-21.97	4.96	-4.43	0.00**	0.66	5.01	0.13	0.90
Flow rate	-37.27	6.74	-5.53	0.00**	-8.47	5.09	-1.66	0.11
pH	13.63	2.43	5.61	0.00**	0.06	1.81	0.03	0.98
Urea	-0.27	0.13	-2.01	0.06	-0.01	0.05	-0.11	0.92
Calcium	2.85	0.56	5.06	0.00**	0.40	0.54	0.75	0.46
Phosphorous	-1.63	0.44	-3.69	0.00**	0.07	0.36	0.21	0.84
Magnesium	6.57	2.38	2.76	0.01*	1.29	2.76	0.47	0.65
PII	21.48	4.08	5.26	0.00**	-1.29	4.43	-0.29	0.77
CaII	-4.44	2.64	-1.68	0.11	-4.16	4.36	-0.96	0.35
GI	3.36	10.33	0.33	0.77	8.49	7.22	1.18	0.25

*Significant (P<0.05)

** Highly Significant (P<0.01)

- Higher salivary lipid peroxidation as reflected by slightly elevated MDA level though with no significant difference among old adults may further explain higher dental caries intensity among them. The possible explanation is that reactive oxygen species might induce lipid peroxidation and damage of the immune cells⁽²⁵⁾ that might affect dental caries process.
- Lower salivary flow rate among old adults though statistical difference was not significant. This is confirmed by inverse highly significant β coefficient for salivary flow rate on DS. Since the washing action of saliva plays an important role in the clearance of food debris and bacteria also its protective constituents increase with increasing flow rate⁽²⁶⁾.
- Changes in lifestyle among elderly that lead to more opportunity for snacking⁽¹⁾.

Special oral health preventive and educational programs are needed for old adults. In addition data gained here might provide clue for the use of salivary antioxidants as a mean for monitoring oral health and success of treatment.

REFERENCES

- Andrews MB, Claypool S, Johnson PH, Mauro E, Weinstock D, Wittig PA. Mastering geriatric care, assessing older adults, managing disorders and complications, avoiding drug dangers, detecting abuse and neglect. Springhouse, Pennsylvania; 1997. p. 1-16.
- Drummond JR, Newton JP, Yemm R. Color atlas and text of dental care of the elderly. Mosby-Wolf, USA; 1995. p. 1-52.
- Silvesterstone LM, Johnson NW, Hardie JM, Williams RAD. Dental caries, aetiology, pathology and prevention. Hong Kong: The Mak Millian Press; 1981. p. 176-7.
- Fejerskov O, Thylstrup A. Different concepts of dental caries and their implications. In: Textbook of clinical cariology ed. By Thylstrup A and Fejerskov O. 2nd ed. Munksgaard, Copenhagen; 1994. p. 209-217.
- Furhoff A, et al. A multidisciplinary clinical study of patients suffering from illness associated with release of mercury from dental restorations. Medical and odontological aspects. Scan J Primary Health Care 1998; 16: 247-52.
- O'Sullivan EA, Curzon ME. Salivary factors affecting dental erosion in children. Caries Res 2000; 34: 82-7.
- Sulaiman AWR. Quantitative measurement of urea content in saliva, acquired pellicle and dental plaque in relation to dental caries susceptibility in human adults. A Ph.D. Thesis. College of Dentistry, University of Baghdad, 2000.
- El-Samarrai SK. Major and trace elements of permanent teeth and saliva among a group of adolescent in relation to dental caries, gingivitis and *Mutans Streptococci*. A Ph.D. Thesis. College of Dentistry, University of Baghdad, 2001.
- Suntsov VG, Antonova AA, Lebed'ko OA, Talovskaia VS. Peculiarities of saliva chemiluminescence and microelement hair content in children with various dental caries activity. Stomatologia (Mosk) 2008; 87(1): 4-7.
- Marquis RE. Oxygen metabolism, oxidative stress and acid-base physiology of dental plaque biofilms. J Ind Microbiol 1995; 15(3): 198-207.
- Tenovuo J, Lagerlöf F. Saliva. In: Textbook of clinical cardiology ed. By Thylstrup A and Fejerskov O. 2nd ed. Munksgaard, Copenhagen; 1994. p. 17-43.
- Silness J, Løe H. Periodontal disease in pregnancy II. Acta Odontol Scand 1964; 24: 747-59.

13. Ramfjord SP. Indices for prevalence and incidence of periodontal disease. *J Periodontol* 1959; 30: 51-9.
14. Löe H, Silness J. Periodontal disease in pregnancy. I. *Acta Odontol Scand* 1963; 21: 533-51.
15. Mühlemann HR. Oral epidemiology-caries. In: *Introduction to oral preventive medicine*. Buch-und Zeitschriften-Verlag, Die Quintessenz (Translated in English); 1976. p. 73-100.
16. Wootton IDP. *Microanalysis in medical biochemistry*. 5th ed. Churchill Livingstone, Edinburgh; 1974. p. 156-9.
17. Varley H. *Practical clinical biochemistry*. 4th ed. The white friars press limited, London and Tonbridge, Great Britain; 1967.
18. Colowick SP, Kaplan NO. *Methods in enzymology*. Vol. 62, part D, Academic press, USA; 1979. p. 7.
19. Beng JA, Aust SD. Estimation of serum malondialdehyde level. In: *Methods in enzymology* Hoffee Jones ed. By Hoffee PA and Jone ME. Academic Press, a Subsidiary of Harcourt Brace Jovanovich Publisher, New York; 1978.
20. Edgar WM. Saliva: its secretion, composition and functions. *Brit Dent J* 1992; 172: 305-12.
21. Schlenker ED. *Nutrition in aging*. 3rd ed. WCB McGraw-Hill Co. 1998. p. 295-325, 148-76, 177-207.
22. Hershkovich O, Shafat I, Nagler RM. Age-related changes in salivary antioxidant profile: possible implications for oral cancer. *J Gerontol. A Biol Sci Med Sci* 2007; 62: 4, 361-6.
23. Steele JG, Walls AWG. Prevention in the ageing dentition. In: *The prevention of oral disease* ed. By Murray JJ, Nunn JH, Steele JG. 4th ed. Oxford, USA; 2003. p. 190-207.
24. Thylstrup A, Fejerskov O. Epidemiology of dental caries. In: *Textbook of clinical cariology*. 1st ed. Munksgarrd, Copenhagen; 1986. p. 266-84.
25. Meydani SN, Barklund MP, Liu S, Meydani M, Miller RA, Cannon JG, et al. Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *Am J Clin Nutr* 1990; 52: 557-63.
26. Mandel ID. The role of saliva in maintaining oral homeostasis. *JADA* 1989; 119: 298-304.