Salivary antioxidants and physicochemical characteristics related to periodontal disease among a group of old adults

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ABSTRACT

Background: Old adults experienced pronounced oral changes. Saliva composition particularly the antioxidants showed 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 periodontal disease 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. Periodontal disease was evaluated by using the gingival index, periodontal pocket depth and clinical attachment level. 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: 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. Salivary constituents (urea, calcium, phosphorous and magnesium) showed no significant difference between the two age groups. Mean gingival index was highly significantly higher among old adults. The extent of pocket depth was higher among old adults at ≥4 and ≥5 mm thresholds but ≥7 mm threshold was absent among them. Clinical attachment level extent was higher among old adults at all thresholds of severity. Salivary albumin revealed inverse significant correlation with severe thresholds of clinical attachment level (i.e. ≥7, ≥8, ≥9 mm). Lipid peroxidation (malondialdehyde) showed positive highly significant correlation with ≥9 mm attachment level threshold among old adults. Salivary flow rate revealed inverse correlation with almost all clinical attachment level thresholds among old adults.

Conclusions: Periodontal disease revealed higher severity among old adults. Salivary antioxidants and physicochemical characteristics were found to affect periodontal health status among old adults.

Key words: Salivary antioxidants, periodontal diseases, old adults. (J Bagh Coll Dentistry 2009; 21(4):103-107)

INTRODUCTION

Aging is the process of gradual and spontaneous changes resulting in maturation through childhood, puberty and young adulthood, and then decline through middle and late age [(1)]. Age-associated changes in periodontal tissues include atrophic and degenerative changes with a more irregular structure of the periodontal ligament and a decrease in fiber and cellular content [(2)]. Periodontal diseases are infections of multifactorial etiology in which bacteria are the main causative agent while environmental and genetic influences affect disease severity [(3)]. Salivary physicochemical characteristics were found to affect periodontal health [(4, 5)]. Also periodontal disease involved a variety of molecular species among them reactive oxygen species. Antioxidants have been shown to play a critical role in modulating reactive oxygen species-induced damage during periodontitis [(6)].

Many controversies were reported concerning the association of salivary physicochemical characteristics with periodontal diseases [(7, 8)]. Also several studies recorded a controversy regarding the correlation between salivary reactive oxygen species, antioxidants, and periodontal disease [(9, 10)]. As far as it is known, there are no previous Iraqi studies concerned with the relation between reactive oxygen species, salivary antioxidants and periodontal diseases among old adults; therefore, it was decided to conduct this study.

MATERIALS AND METHODS

The studied sample 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 [(11)]. 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

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Tenovuo and Lagerlöf [12]. 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.

Periodontal disease was evaluated by using the gingival index (GI) [13], periodontal pocket depth (PPD) and clinical attachment level (CAL) [14]. Salivary antioxidants were determined by photometric methods. Some were measured by manual methods as in case of total protein using Biuret method [15], vitamin E depending on Emmerie-Engel reaction [16], vitamin C by using 2, 4-dinitrophenyl hydrazine (DNPH) method [17] and salivary MDA using the method of Beng and Aust [18]. 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

RESULTS

Salivary antioxidants (total protein, albumin, vitamin E and vitamin C) recorded lower mean values among old adults with significant difference for vitamin C only ($t=2.37$, $P<0.05$, $df=68$). 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 ($P>0.05$). It was found that mean GI was higher among old adults (1.17±0.08) compared with middle-aged ones (0.10±0.29) with highly significant difference ($t=-3.40$, $P<0.01$, $df=68$).

The extent (mean percentage of sites) of periodontal pocket depth was highly significantly higher at ≥4 mm threshold among old adults ($t=-2.74$, $P<0.01$, $df=68$), while ≥7 mm threshold was absent among them. No cases with ≥6 mm PPD threshold was found in both age groups (Table 3). Table 4 reveals that the extent of clinical attachment level was higher among old adults at all thresholds. The correlation coefficients of attachment level thresholds with salivary antioxidants and MDA are shown in Table 5. Regarding old adults, albumin showed weak negative significant relations with ≥7, ≥8 and ≥9 mm thresholds. On the other hand, MDA showed strong positive highly significant correlation with ≥9 mm threshold. Table 6 reveals that among old adults salivary flow rate showed weak negative highly significant relations with ≥1 and ≥ 6 mm threshold and weak negative significant relations with ≥2, ≥3, ≥4 and ≥5 mm thresholds.

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 [19]. 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 reduced masticatory performance [20]. Another explanation is the elevated free radical generation with aging so salivary antioxidants would be exhausted in reaction with the elevated free radicals [21].

The present investigation revealed higher gingival inflammation among old adults with highly significant difference. Also old adults revealed higher attachment level extent among old adults at all thresholds. This goes in accordance with previous studies [22, 23]. Regarding pocket extent, ≥ 4 mm threshold was highly significantly higher among old adults but ≥ 7 mm threshold was absent among them compared with middle-aged. So the age difference in pocket depth is less clear this might be related to the fact that pocket depth is a variable measure while attachment loss represents a precise measure of overall periodontal disease experience [22]. Higher gingival inflammation and attachment level among old adults as seen by the current study might be related to:

1. Highly significantly higher gingival inflammation among old adults may explain their higher attachment level since gingivitis is usually a precursor for periodontitis [24].
2. Lower salivary antioxidants level (including total protein, albumin, vitamin E, and vitamin C) among old adults since these antioxidants enhance periodontal health by providing protection against ROS-induced damage of periodontal tissues [25]. This is supported by the inverse significant correlation of albumin with severe thresholds of clinical attachment level (i.e. ≥7, ≥8 and ≥9 mm).

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: Extent (Mean percentage of sites ±S.D.) of periodontal pocket depth according to different thresholds of severity among old adults and middle-aged.

PPD (mm)	Old adults (55-65 years)			Middle-aged (30-40 years)			Statistical difference		
	No.	Mean	±SD	No.	Mean	±SD	t-test	df	P-value
≥4	35	1.90	2.86	35	0.48	1.08	-2.74	68	**0.00****
≥5	35	0.28	0.70	35	0.12	0.49	-1.14	68	0.26
≥7	35	0.00	0.00	35	0.12	0.49	1.44	68	0.16

****Highly Significant (P<0.01)**

Table 4: Extent (Mean percentage of sites ±S.D.) of clinical attachment level according to different thresholds of severity among old adults and middle-aged.

CAL (mm)	Old adults (55-65 years)			Middle-aged (30-40 years)			Statistical difference		
	No.	Mean	±SD	No.	Mean	±SD	t-test	df	P-value
≥1	35	63.17	27.48	35	20.57	25.24	-6.75	68	**0.00****
≥2	35	41.23	32.29	35	6.10	13.97	-5.77	68	**0.00****
≥3	35	28.38	27.31	35	1.83	4.75	-5.67	68	**0.00****
≥4	35	20.96	21.82	35	0.59	2.45	-5.49	68	**0.00****
≥5	35	15.33	17.16	35	0.42	1.72	-5.12	68	**0.00****
≥6	35	9.84	12.60	35	0.18	0.74	-4.53	68	**0.00****
≥7	35	2.33	2.85	35	0.12	0.49	4.53	68	**0.00****
≥8	35	0.63	1.18	35	0.12	0.49	-2.37	68	**0.02***
≥9	35	0.14	0.38	35	0.00	0.00	-2.09	68	**0.04***

***Significant (P<0.05) * Highly Significant (P<0.01)**

Table 5: Correlation coefficients of attachment level extent according to different thresholds of severity with salivary antioxidants and lipid peroxidation biomarker among old adults and middle-aged.

Age group	CAL (mm)	Total protein		Albumin		Vitamin E		Vitamin C		Uric acid		MDA	
		r	P	r	P	r	P	r	P	r	P	r	P
Old adults (55-65years)	≥ 1	-0.05	0.78	-0.21	0.23	0.09	0.63	0.10	0.57	0.06	0.76	0.05	0.76
	≥2	-0.18	0.29	-0.24	0.17	0.11	0.52	-0.18	0.30	-0.04	0.83	0.25	0.14
	≥3	-0.09	0.59	-0.18	0.30	0.18	0.29	-0.18	0.30	-0.16	0.35	0.21	0.22
	≥4	-0.05	0.80	-0.16	0.38	0.21	0.23	-0.17	0.34	-0.22	0.21	0.16	0.37
	≥5	0.01	0.95	-0.11	0.55	0.24	0.16	-0.16	0.37	-0.30	0.08	0.14	0.43
	≥6	0.08	0.64	-0.02	0.91	0.34	**0.04***	-0.14	0.44	-0.31	0.07	0.16	0.38
	≥7	-0.15	0.38	-0.38	**0.02***	0.07	0.69	-0.14	0.41	-0.17	0.32	0.22	0.21
	≥8	-0.21	0.22	-0.43	**0.01***	0.004	0.98	-0.09	0.60	-0.05	0.76	0.13	0.45
	≥9	-0.13	0.46	-0.36	**0.04***	0.01	0.97	-0.06	0.73	0.22	0.21	0.68	**0.00****
Middle-aged (30-40 years)	≥ 1	-0.12	0.48	0.29	0.09	-0.03	0.86	-0.01	0.94	-0.06	0.73	0.27	0.13
	≥2	-0.06	0.72	0.37	**0.03***	-0.08	0.66	-0.02	0.90	-0.12	0.51	0.17	0.32
	≥3	-0.20	0.24	0.13	0.45	-0.05	0.77	-0.14	0.44	-0.02	0.90	0.28	0.10
	≥4	-0.19	0.27	0.01	0.95	-0.05	0.77	-0.10	0.55	0.01	0.96	0.30	0.08
	≥5	-0.19	0.27	0.01	0.95	-0.05	0.77	-0.10	0.55	0.01	0.96	0.30	0.08
	≥6	-0.19	0.27	0.01	0.95	-0.05	0.77	-0.10	0.55	0.01	0.96	0.30	0.08
	≥7	-0.19	0.27	0.01	0.95	-0.05	0.77	-0.10	0.55	0.01	0.96	0.30	0.08
	≥8	-0.19	0.27	0.01	0.95	-0.05	0.77	-0.10	0.55	0.01	0.96	0.30	0.08
	≥9	-	-	-	-	-	-	-	-	-	-	-	-

Significant (P<0.05)** * Highly Significant (P<0.01)**

Table 6: Correlation coefficients of attachment level extent according to different thresholds of severity with salivary physicochemical characteristics among old adults and middle-aged.

Age group	CAL (mm)	Flow rate		PH		Urea		Calcium		Phosphorous		Magnesium	
		r	P	r	P	r	P	r	P	r	P	r	P
Old adults (55-65years)	≥ 1	-0.44	**0.00****	-0.42	**0.01***	-0.06	0.73	0.16	0.36	-0.05	0.79	-0.41	**0.02***
	≥2	-0.34	**0.04***	-0.41	**0.01***	0.23	0.19	0.53	**0.00****	0.29	0.09	-0.09	0.59
	≥3	-0.34	**0.04***	-0.46	**0.00****	0.09	0.61	0.38	**0.03***	0.12	0.49	-0.04	0.84
	≥4	-0.38	**0.03***	-0.46	**0.00****	0.07	0.70	0.35	**0.04***	0.09	0.60	-0.00	0.99
	≥5	-0.40	**0.02***	-0.44	**0.00****	-0.02	0.92	0.26	0.14	0.04	0.83	0.09	0.60
	≥6	-0.44	**0.00****	-0.42	**0.01***	0.06	0.74	0.28	0.10	0.08	0.64	0.22	0.20
	≥7	-0.20	0.24	-0.37	**0.03***	-0.12	0.50	0.39	**0.02***	0.02	0.90	-0.15	0.41
	≥8	0.15	0.41	-0.16	0.35	-0.30	0.08	0.23	0.18	-0.18	0.30	-0.24	0.17
	≥9	-0.08	0.67	-0.04	0.84	0.17	0.34	0.28	0.10	0.33	0.053	0.21	0.22
Middle-aged 30-40 years)	≥ 1	0.14	0.43	0.31	0.07	0.29	0.09	-0.09	0.59	0.26	0.14	-0.03	0.87
	≥2	0.32	0.06	0.12	0.48	0.37	**0.03***	-0.11	0.53	0.14	0.42	-0.13	0.45
	≥3	0.18	0.30	0.22	0.20	0.32	0.06	-0.24	0.16	0.20	0.25	-0.09	0.60
	≥4	0.07	0.68	0.22	0.22	0.26	0.13	-0.26	0.13	0.19	0.28	-0.04	0.82
	≥5	0.07	0.68	0.22	0.22	0.26	0.13	-0.26	0.13	0.19	0.28	-0.04	0.82
	≥6	0.07	0.68	0.22	0.22	0.26	0.13	-0.26	0.13	0.19	0.28	-0.04	0.82
	≥7	0.07	0.68	0.22	0.22	0.26	0.13	-0.26	0.13	0.19	0.28	-0.04	0.82
	≥8	0.07	0.68	0.22	0.22	0.26	0.13	-0.26	0.13	0.19	0.28	-0.04	0.82
	≥9	0.07	0.68	0.22	0.22	0.26	0.13	-0.26	0.13	0.19	0.28	-0.04	0.82

Significant (P<0.05)** * Highly Significant (P<0.01)**

3. Slightly higher lipid peroxidation (i.e. MDA) among old adults though with no significant difference. This is supported by the positive highly significant correlation of salivary MDA with severe threshold of attachment level

(≥9 mm) among old adults. Since reactive oxygen species damage periodontal tissues by causing lipid peroxidation of the cell wall and so cell death [9].

4. Reduction of salivary flow rate with no significant difference among old adults might exacerbate periodontal disease among them. This is supported by its inverse correlation with almost all CAL thresholds among old adults.

Old adults are considered as an important target group with special oral health needs. Salivary antioxidant level could be used as a mean for monitoring oral health and success of treatment during the periodontal maintenance period.

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