Journal of Dental Sciences (2014) 9, 85—90

Available online at www.sciencedirect.com

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journal homepage: www.e-jds.com

ORIGINAL ARTICLE

Age-related changes in salivary biomarkers

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Received 26 September 2013; Final revision received 26 October 2013
Available online 6 February 2014

KEYWORDS
aging;
alpha-amylase;
calcium;
reduced glutathione: oxidized glutathione ratio;
saliva

Abstract *Background/purpose:* Saliva plays a critical role in the oral cavity health; the levels of its constituents alter with age. The reduced glutathione:oxidized glutathione ratio (GSH:GSSG) in the plasma is reported to be lower in elderly people and thus can be an indicator of age. The aim of this study was to detect age-related changes in salivary biomarker levels and evaluate whether the salivary GSH:GSSG ratio can be an indicator of aging.
Materials and methods: Individuals who participated in this study were divided into two groups: the elderly group ($n = 20$; age 60—80 years) and the young group ($n = 20$; age 20—30 years). Unstimulated saliva was collected passively for 5 minutes, followed by clinical examination. The salivary flow rate (SFR), pH, and buffering capacity were measured, followed by centrifugation of saliva, collection of supernatant, and measurement of the following biomarkers: calcium (Ca), alpha (α)-amylase, GSH, GSSG, matrix metalloproteinase-8 (MMP-8), collagenase type-I, and tissue inhibitor of metalloproteinase (TIMP-1). Descriptive analyses of variables were performed.
Results: The elderly group showed significantly lower SFR and Ca than the young group, whereas collagenase type-1 and MMP-8 were significantly lower in the young group. None of pH, buffering capacity, α-amylase, GSH, GSSG, GSH:GSSG, or TIMP-1 showed any statistically significant difference between the two groups.
Conclusion: Saliva is a mixture of components, the levels of which can increase, decrease, or remain stable with age. Although the GSH:GSSG ratio was lower in the elderly group, it did not reach a level of significance.

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1991-7902/$36
http://dx.doi.org/10.1016/j.jds.2013.11.002

Introduction

The number of people aged over 60 years is growing faster than that of any other age group. Approximately 10% of the world's population is over the age of 60 years; this number is likely to increase to 15% by the year 2050.[1] Physiological changes occur with aging in almost all organ systems.[2] It is widely accepted that saliva composition alters with age,[3] which can manifest as low resistance of the elderly to oral diseases.[4] Saliva is an essential fluid that maintains oral health and homeostasis by providing the necessary host defense functions.[5] This fluid can be collected non-invasively and used as a diagnostic tool to provide information about the health or disease status of an individual.[6] Despite this knowledge, the literature is controversial regarding the age-related changes in the salivary biomarker.

Among the many proposed theories of aging, free radical and oxidative stress theories received special attention.[7] Glutathione is the most prevalent and most important intracellular thiol–disulfide redox buffer in mammalian cells; it exits in two forms: reduced glutathione (GSH) and oxidized glutathione (GSSG). GSH, the active form, is a water soluble tripeptide containing cysteine, glutamic acid, and glycine[8,9]; it accounts for around 90% of the intracellular thiols. The remaining 10% is made up of other small thiol compounds.[10] The thiol group in cysteine is a potent reducing agent, rendering GSH an essential antioxidant in the detoxification of a variety of electrophilic compounds and peroxides.[11] It is found in micromolar (μM) concentrations in body fluids and in millimolar (mM) concentrations in tissue. The GSH:GSSG ratio is indicative of oxidative stress and cellular health.[12] It was reported that the GSH:GSSG ratio in the plasma is an indicator of aging.[13]

Calcium (Ca) is the fifth most abundant mineral in the human body and one of the most intensively studied minerals in saliva due to its role in dental and gingival health.[14,15] Serum Ca ions were reported to be lower in healthy elderly persons than in healthy young adults.[16] Alpha (α)-amylase is one of the most abundant components in human saliva that can play a role in oral health or disease. In solutions this enzyme binds to bacteria, contributing to the bacterial clearance. By contrast, in enamel pellicle, it initiates the digestion of starch, thus providing substrates for colonizing bacteria and enhancing their adhesion to tooth surfaces.[17,18] Both functions need an intact enzyme conformation.[17] The changes in salivary Ca and α-amylase levels with age are still unclear.

To our knowledge, no existing studies have addressed the effect of aging on the GSH:GSSG ratio in saliva. Thus, the aim of this study was to evaluate the changes in the levels of certain markers of unstimulated saliva with age. The tested null hypothesis was that no difference was observed in the levels of salivary biomarkers between the elderly and young groups.

Materials and methods

This study was carried out in 20 healthy young adults in the age group of 20–30 years and 20 healthy elderly people in the age group of 60–80 years, recruited from the Tokyo Medical and Dental University. Informed consent was obtained from all the study participants, and the study protocol was approved by the ethical committee of the Tokyo Medical and Dental University (number 701).

Prior to saliva collection, patients were allowed to rest for a few minutes and asked to rinse their mouth with water. They were seated comfortably, and asked to lean slightly forward and not to swallow. After 5 minutes, whole saliva was collected in a collection tube by passive drooling. Clinical examination was performed to record the number of decayed, missing, and filled teeth (DMFT).

The amount of saliva was measured and the salivary flow rate (SFR) was calculated (mL/5 minute). The pH and buffering capacity of saliva were measured immediately using a hand-held pH meter (Checkbuf; Horiba Ltd., Kyoto, Japan), followed by centrifugation of saliva for 10 minutes at 10,000 rpm (9,170 × *g*). The supernatant was collected and stored at −80°C until further analysis of the following salivary biomarkers: Ca (AA-630, an atomic absorption spectrometer; Shimadzu Corporation, Kyoto, Japan), α-amylase (EnzyChrom, an α-amylase assay kit; BioAssay Systems, Hayward, CA, USA), GSH and GSSG (GSSG/GSH Quantification Kit; Dojindo Laboratories, Kumamoto, Japan), matrix metalloproteinase-8 (MMP-8; Quantikine Human Total MMP-8 ELISA Kit; R&D Systems Inc., Minneapolis, MN, USA), collagenase type-I (Type-I Collagenase Assay Kit; Primary Cell Co., Ltd., Sapporo, Japan), and tissue inhibitor of metalloproteinase (TIMP-1; Quantikine Human TIMP-1 Immunoassay; R&D Systems Inc.). Descriptive analyses of variables were performed. Variables of two groups were compared using Student *t* test, and the correlation was assessed using Pearson coefficient.

Results

Forty healthy individuals were divided into two groups according to their age. Table 1 represents the mean age and DMFT of each group. Table 2 shows the mean and the standard deviation of each evaluated salivary variable in the individuals tested. The SFR was statistically lower in elderly people ($P < 0.001$), whereas saliva pH ($P = 0.098$) and buffering capacity ($P = 0.594$) showed no statistically significant difference between the two groups. Neither the GSH ($P = 0.599$) nor the GSSG ($P = 0.571$) level showed a statistically significant difference between elderly and young adults. Although the GSH:GSSG ratio was higher in young individuals, it was not statistically significant

Table 1 Distribution of participants and DMFT in different age groups.

Age group	*N*	Mean age, y	DMFT
Young group	20	27.8 ± 2.6[a]	7.7 ± 6.5[a]
Elderly group	20	68.6 ± 7.4[b]	22.2 ± 5.5[b]

Data are presented as mean ± SD.
DMFT = decayed, missing, and filled teeth; SD = standard deviation.
[a,b] Different letters represent a significant difference ($P < 0.05$).