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Zinc Salivary Levels in Healthy Individuals of Bulgarian Population

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The main goal of this study is to assess zinc (Zn) salivary levels in healthy Bulgarian individuals. Totally 40 healthy volunteers have been investigated – 31 males and 9 females. Zn is measured in morning saliva collected in Saliveti tubes, Sarstedt. After centrifugation, the saliva is diluted 1: 2 with 1%HNO_3 and Zn is measured by flame atomic absorption spectrometry (AAnalyst 400, Perkin-Elmer, with deuterium correction). Mean Zn salivary levels are 1.43 ± 0.57μmol/ L for all studied individuals and higher levels in females (1.76 ± 0.56 μmol / L) than males (1.36 ± 0.55 μmol/L) with no significant difference (p = 0.076). Our previous studies establish significantly higher serum Zn levels in females (13.09 ± 2.25 μmol/l) than in males (12.45 ± 3.58 μmol/l). It seems likely that salivary Zn levels follow similar biological gender variation, as serum levels do.

Key words: saliva, zinc, salivary omics

Introduction

Saliva is composed of salivary glands secrets, cellular debris, upper respiratory tract fluid and microorganisms in the oral cavity [3]. Saliva testing for monitoring and diagnosis of both oral and systemic disorders is a challenge in accordance with the contemporary development of "omics" sciences. Research applications are directed to dental diseases and evaluation of hormonal, neurological and emotional status, and human behavior [2, 3]. Essential micronutrients copper (Cu) and zinc (Zn), simultaneously with salivary cortisol and other laboratory findings as adrenocorticotropic hormone, catecholamines and various cytokines, could be useful stress biomarkers [3]. Zn with neurotransmission and receptor functions is involved in mental health [2]. Cu and Zn are suggestive to participate in sleep durations due to the fact that they are antagonists of the sleep-mediated N-methyl-D-aspartate glutamate (NMDA) receptor [12]. Systemic zinc supplementation may alter salivary stress hormone levels, particularly these of cortisol [2].

The interest on saliva as a biological specimen is provoked by non-invasive way of collection and large enough sample volume [3]. Analysing of salivary samples could serve as an aspect in laboratory assessment of hormonal, emotional and immunological status and also dental health. Knowledge on the metabolic relations of trace elements in different body fluids could be of benefit in medicine. Specific salivary pathological findings could be compared to those in other fluid matrixes. "Omics" development of science and, in particular salivary omics (genomics, proteomics and metabolomics) is associated with the introduction of new biomarkers in clinical practice [9]. In this sense, salivary metallomics is definitely a modern perspective.

Zinc has a dual nature in human body. It is essential for life as component of various metalloenzymes with physiological significance for immune system, cell division and growth processes, reproductive system, etc. From the other hand, the element is cytotoxic when accumulated in high concentrations [6, 8]. Data about salivary levels of Cu, Zn and Fe as biomarkers in oral carcinogenesis are provided [11]. Recent observations underline the significance of trace elements in saliva, particularly Cu, Zn and Fe, as markers for the effects of the social stress [10].

The main goal of this study is to evaluate salivary zinc levels in healthy individuals of Bulgarian population.

Materials and Methods

The study comprises 40 volunteers (males:females = 31:9) with no evidence of anemia and impaired glucose tolerance. A signed informed consent form of all participants has been obtained. Non-stimulated salivary samples are collected in Salivette tubes-Sarstedt, after 2-hours pause in food and liquid intake and cigarette smoking prior to the saliva release. The samples are centrifuged at 2500 rpm for 10 min at room temperature and the supernatants are stored at -80°C until the analyses. Saliva samples are diluted 1:2 with 1% nitric acid immediately before the measurement. Zn levels are measured by flame atomic absorption spectrophotometry (AAnalyst 400, Perkin-Elmer) with a deuterium background corrector. All samples are determined in one run to minimize the analytical variation. The analyses are performed in Clinical Laboratory of St. Ivan Rilski University Hospital, Medical University of Sofia, in 2017. The results are statistically processed by MED CALC program.

Results and Discussion

Zn levels in saliva are presented in **Table 1**. Data are not normally distributed in both groups of males and females.

Table 1. Descriptive statistics and distribution analyses

Zn Saliva µmol/L	**Gender**								**Total**			
	Males				**Females**							
	n	Mean	SD	Distribution	n	Mean	SD	Distribution	n	Mean	SD	Distribution
	31	1.36	0.55	0.0038	9	1.76	0.56	<0.0001	40	1.43	0.57	0.0345

The established mean values for zinc in saliva are 1.43±0.57 μmol/L. Comparing to previously published results of other authors, zinc concentrations in the present study are the lowest (**Table 2**). The reasons for this observation could be multifactorial: diets, soil contents, age of participants or analytical variations.

Table 2. Salivary Zn concentration in comparison to the data of other studies

	Present study, 2017	**Wang, D. et al., 2008 [13]**	**Erkekoglu, P. et al, 2016 [2]**	**Mehmetoğlu, I. et al., 2013 [7]**	**Jassim A. M. N. etal., 2016 [5]**
Zn Saliva μmol/L	1.43±0.57	3.97±2.019	2.57±1.90	4.36±3.36	16.24±0.73
Method for determination	AAS	ICP-MS	Colorimetric	AAS	AAS

- AAS – Atomic absorption spectrophotometry
- ICP-MS – Inductive Coupled plasma mass spectrometry

The Mann-Whitney test points no statistically significant difference between the levels of salivary Zn depending on gender: p=0.0776. Higher levels are established in females than in males. One limitation of the present study is the limited numbers of the included individuals. In any case, eventual gender variation must be confirmed by enough numbers of individuals in both studied groups. Gender and age are widely known biological factors for variation of trace element levels in human body [8]. Our previous observations for Bulgarian population reveal higher serum Zn levels in women (13.09±2.25 μmol/l) than in men (12.45±3.58 μmol/l) with significant statistical difference ($p < 0.01$) [4]. It seems likely that the salivary levels follow the same model for gender distribution.

Salivary diagnostics becomes more and more a reality nowadays. But it must face to certain challenges: standardization of preanalytical phase; measurement with acceptable sensitivity, establishment of factors for variability that define biochemical profile of saliva with potential for clinical use.

Conclusion

Saliva as a specimen in clinical laboratory practice might complement and enrich the disease management.

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