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## Salivary Electrolytes in Patients with Periodontal Disease.

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### ABSTRACT

It is well known that periodontal disease is among others consequence of bacterial invasion of the host periodontal tissues. Saliva has an important role in antibacterial defence within oral cavity and even further on. Some of these antibacterial activities are probably dependent on certain salivary electrolytes such as copper, magnesium, etc. Therefore the aim of this study was to determine levels of salivary phosphate, copper, chloride, potassium and sodium in subjects with and without periodontal disease in order to explore its potential diagnostic implications. This study included 35 patients with periodontal disease and 41 controls. The levels of sodium, potassium and chloride were determined by indirect potentiometry, copper was determined by atomic absorption spectrophotometry, and levels of magnesium were determined by spectrophotometric method with xylydyl blue. Parametric statistics was used for data analysis. No significant difference in any of the measured periodontal clinical parameters was found between patients with chronic and aggressive periodontitis. No significant difference in salivary phosphate, sodium, potassium, and chloride between patients and controls were found. Conclusions: Patients with periodontitis had significantly higher salivary copper concentration compared to the control group which might reflect either increased antimicrobial activity or ineffective enzymatic activity various copper containing enzymes.

**Keywords:** saliva, electrolytes, chronic periodontitis, aggressive periodontitis.

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## INTRODUCTION

Periodontal disease has certain genetic background, but also local factors such as smoking and neglected oral hygiene as well as systemic ones such as diabetes, cardiovascular disorders, etc. are perpetuators of the disease (1). Periodontal disease starts by bacterial invasion at the interface between the tooth surface and marginal gingiva that induces a local inflammatory response (2). The inflammatory cells release metalloproteinases which degrade gingival collagenous fibrous tissue and eventually the surrounding bone (3). Saliva is an important fluid which due to its constituents has great antioxidant capacity in neutralizing free radicals. Saliva contains enzymatic (superoxide dismutase, glutathione peroxidase, peroxidase, etc.) and non-enzymatic antioxidants (uric acid, albumin, glutathione, vitamins A and C, etc.) (4). Salivary electrolytes take part in the antimicrobial functions of saliva. For example, copper is a part of histatin, antimicrobial salivary peptide, i.e. antibiotic metallopeptide. It is also known that copper is part of superoxide dismutase which has antimicrobial activity and lysyl oxidase which mediates the final step in the collagen synthesis. Additionally, copper is a well-known predictor of bone health (5). Function of many factors involved in wound healing signalling cascade are dependent on their interaction with copper such as glycyl-L-histidyl-L-lysine, tripeptide with high affinity for copper ions within human plasma, stimulation of angiogenesis through multiprotein complexes containing S100A13 protein, induction of vascular endothelial growth factor, expression of integrin and stabilization of fibrinogen (6,7). Tamura and Ochiai suggested that zinc and copper ions markedly enhanced the adhesion and accumulation of salivary and serum proteins on cells of *Porphyromonas gingivalis* and inhibited the coaggregation and hemagglutination of *P. gingivalis*. These cations might be useful for limiting the settlement of *P. gingivalis* in the gingival sulcus with the goal of preventing periodontal disease (8). It is known that magnesium (Mg) is a cation involved in many physiological processes, and that its imbalance is associated with many pathological conditions (9). Magnesium ions in the saliva are necessary for the action of hyaluronidase inhibitors which inhibits bacterial hyaluronidases (10). Mahmood and Shukri reported that Mg is reduced in patients with periodontal disease due to the inflammatory response resulting from bacterial challenge (11). Zaïchk and Bagirov reported that in grave periodontal disease the concentrations of the major electrolytes were increased by 2.3 to 6.6 times on an average, of nitrogen twofold, of scandium, manganese, and chromium by 6.8-8.8 times, and of iron, cobalt, copper, selenium, bromine, silver, and mercury by 1.6-1.9 times (12).

Due to very heterogeneous data on salivary composition in periodontitis, the aim of this study was to compare salivary biochemical composition between patients with periodontitis and healthy controls. Therefore, this study aimed to explore potential diagnostic implications of salivary electrolytes measurements in patients with periodontitis.

## MATERIALS AND METHODS

Prior to this investigation all the participants signed informed consent which was approved by the Ethical Committee of the School of Dental medicine, University of Zagreb. This study was conducted at School of Dental medicine, University of Zagreb and at Laboratory for Enzymatic Analysis, Zagreb University Hospital Center. The study group consisted of 35 patients with chronic and aggressive periodontitis, and the control group consisted of 41 healthy participants. The sample of patients with periodontitis was randomly selected from patients referred to the Department of Periodontology.

Clinical measurements and non-stimulated whole saliva samples were obtained. The diagnostic criteria were defined according to the classification developed at the International Workshop for a Classification of Periodontal Diseases and Conditions in 1999 (13). The inclusion criteria for the study group were: systemically healthy patients diagnosed with generalized aggressive or chronic periodontitis and previously untreated patients. The exclusion criteria for the study group were: participation of patients in other clinical studies, pregnancy, general disorders that compromise the immune response, patients with diabetes mellitus, patients on any immunosuppressive, chemotherapy or corticosteroid therapy, patients with any other inflammatory diseases of the oral cavity, antibiotic therapy in the last three months. The inclusion criteria for the control group were healthy oral mucosa, no sites with PD >3 mm, no radiographic bone loss, and no bleeding on probing on more than 2 teeth. Clinical measurements were taken by a single calibrated examiner with a standard periodontal probe (PCP 15, Hu-Friedy, Chicago, IL, USA). Pocket probing depth (PD), distance between cement-enamel junction and the bottom of the pocket/sulcus (CAL), were measured at six sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual), excluding third

molars. Measurements were recorded to the nearest millimeter. Visible plaque accumulation (PI), presence or absence of plaque on all four tooth surfaces was recorded for each tooth (14). Bleeding on probing (BOP), presence or absence of bleeding up to 15 seconds after gentle probing, was recorded for buccal and lingual surfaces (15). The percentage of surfaces with positive BOP and PI were calculated. Non-stimulated whole saliva was collected between 9 and 12 AM on the day following periodontal measurements, while participants were sitting and spitting into calibrated test tubes (0.2 ml) during five minutes according to Navazesh et al. (16). Saliva specimens were frozen at -20 °C and the analysis was performed in the same week when samples were obtained. The levels of sodium, potassium and chloride were determined by indirect potentiometry on the automatic biochemical analyser Roche Cobas C51 (Roche, Germany). Copper was determined by atomic absorption spectrophotometry (Perkin Elmer Analyst 800). The unit of measurement of electrolytes was µmol/L.

Data were analyzed with statistical software package SPSS v.21.0 (SPSS Inc., Chicago, IL, USA). Normality of distribution was assessed by Kolmogorov Smirnov test. As the data were normally distributed, parametrical statistics was used for analysis. Data were presented as mean ± standard deviation (mean ± SD). To assess inter-group differences, independent samples t-test was used for numerical variables and chi-square test was used for nominal variables. To assess correlation between variables, Pearson’s correlation coefficient was used. Statistical significance was set at 0.05 (p<0.05).

**RESULTS**

Thirty five patients with periodontitis (23 females and 12 males) and 41 controls (32 females and 9 males) participated in the study. Average age of the participant was 46.4 ± 17.2. No differences in sex, age and smoking were found between patients and control group (Table 1). No significant difference in any of the clinical parameters was found between patients with chronic and aggressive periodontitis (Table 2). No significant difference in salivary phosphate, sodium, potassium, and chloride between patients and controls were found. Patients with periodontitis had significantly higher salivary copper concentration compared to control group (Figure 1). No significant differences in salivary phosphate, sodium, chloride and copper were found between patients with chronic and aggressive periodontitis (Figure 2); however there was significant difference in potassium level between two studied groups. A significant correlation between CAL and salivary sodium was also found in patients with periodontitis.

**Table 1: Sociodemographic data of all participants**

	Patients	Controls	p value
Sex (N; %)			
Female	23; 65.7%	32; 78%	0.231
Male	12; 34.3%	9; 22%	
Age (mean ± SD)	45.3 ± 11.6	47.3 ± 21.1	0.614
Smoking (N; %)			
Yes	8; 22.9%	9; 22%	0.925
No	27; 77.1%	32; 78%	

**Table 2: Clinical data regarding periodontal condition of patients with periodontitis**

	Chronic periodontitis	Aggressive periodontitis	p value
PI (mean ± SD)	71.1 ± 27.7	73.3 ± 19.9	0.788
BOP (mean ± SD)	67.9 ± 33.1	84.6 ± 17.2	0.062
% of pockets (mean ± SD)			
1-3 mm	55.7 ± 17.6	57.4 ± 23.9	0.819
4-5 mm	27.1 ± 10.7	26.2 ± 13.7	0.846
>6 mm	17.9 ± 15.3	15.9 ± 19	0.744
CAL (mean ± SD)	3.9 ± 1	4.2 ± 1.2	0.522

PI – plaque index, BOP – bleeding on probing, CAL – clinical attachment level.

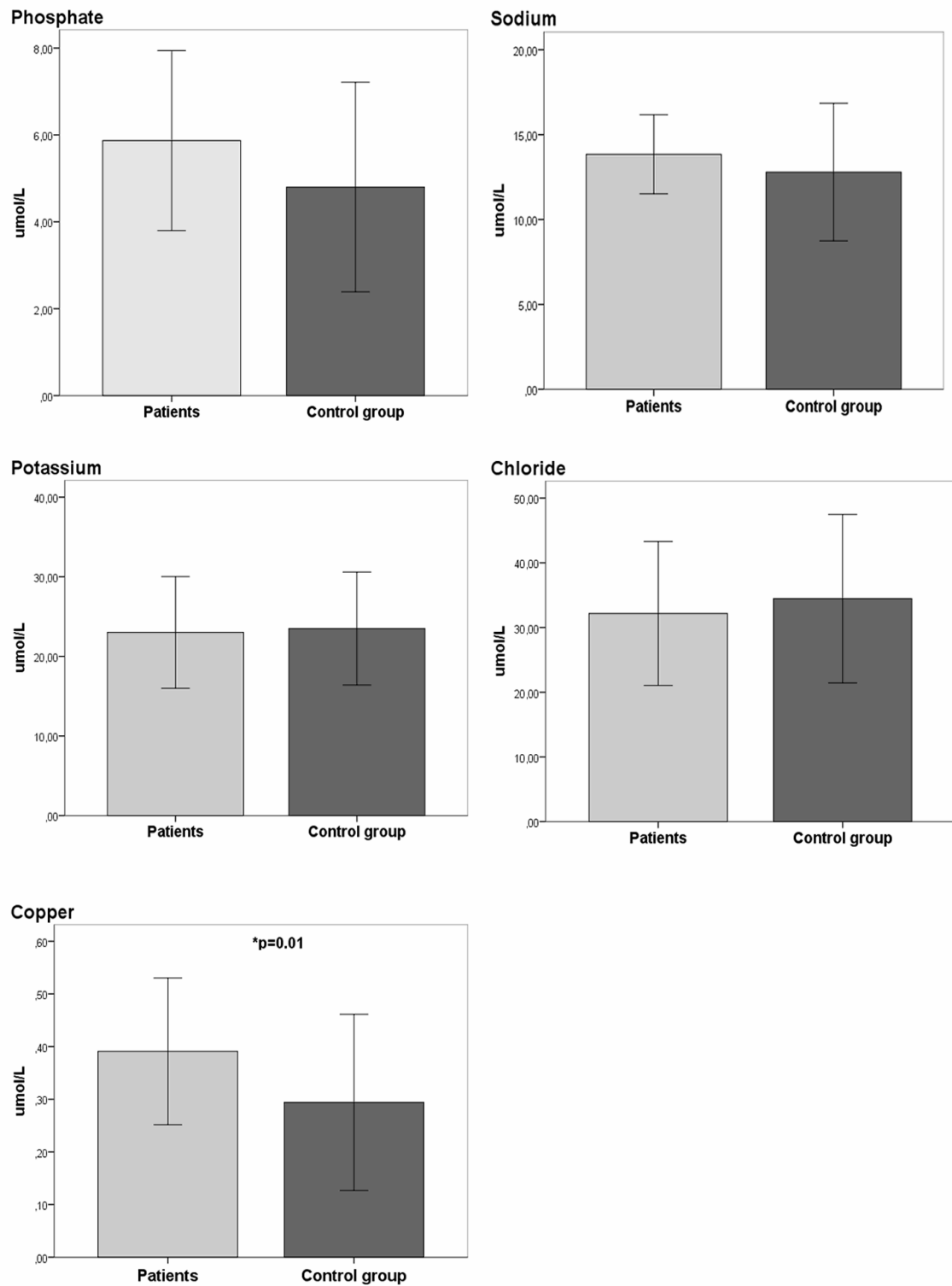


Figure 1: Salivary electrolytes in periodontitis patients and controls.

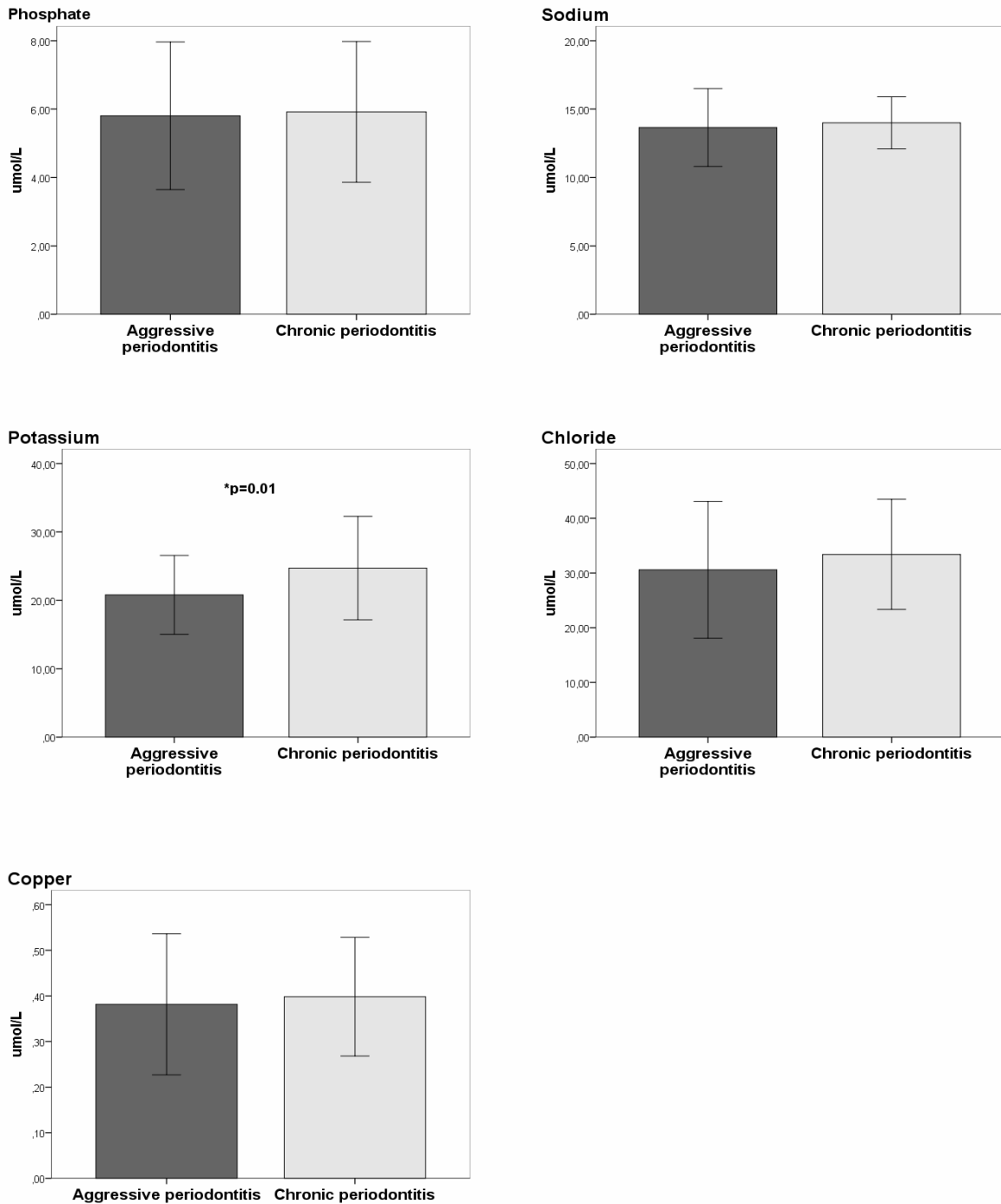


Figure 2: Salivary electrolytes in patients with chronic and aggressive periodontitis.

### DISCUSSION

The results of this study show significant difference in salivary copper between patients with periodontal disease and healthy controls. There are several possibilities which might explain this finding. For example, level of salivary copper was increased in patients with periodontal disease in comparison to the control group. Copper and zinc are components of antibacterial enzyme called superoxide dismutase (SOD) which has an antioxidant potential. Salivary histatins are composed also of copper and have antimicrobial activity within oral cavity (17). Copper enhances the adhesion and accumulation of salivary and serum proteins to *P. gingivalis*, and it is thought that copper might be useful in preventing periodontal disease (8). Therefore, increased salivary copper levels might indicate that either SOD and/or histatins are not working well, so the

copper is found in excess. On the other hand it might be that due to periodontal disease level of salivary copper is increased in saliva of those patients indicating increased antimicrobial activity.

Smoking is considered a major risk factor for the development and progression of periodontal diseases as it is known that smoking is a major source of free radicals within oral cavity. There has been a lot of speculation whether smoking and periodontal disease affect salivary electrolytes. Published data are controversial. According to Zuabi et al. patients with periodontal disease who were smokers had reduced sodium levels in comparison to the non-smokers (18). This is in accordance with results of Kolte et al. who reported reduced concentrations of total proteins, calcium, magnesium and phosphorus in whole saliva in smokers with chronic periodontitis (19). Erdemir et al. found no differences in salivary sodium levels between smokers and non-smokers with periodontal disease (20). The results of our study show that salivary sodium levels were increased in patients with periodontal disease who were smokers in comparison to the non-smokers. Additionally, clinical attachment loss correlated with salivary sodium levels may thus indicate that sodium levels are increased due to attachment loss and sodium leakage from gingival crevicular fluid. It is also possible that elevated sodium levels are result of release from connective tissue or bone due to attachment loss. Our results are also in concordance with the ones of Grossi and co-workers who studied the effect of cigarette smoking on the attachment apparatus and alveolar bone height; heavy smokers had greater odds ratio for both attachment loss and alveolar bone loss when compared with non-smokers (21). Erdemir et al. (20) also reported positive correlations between the levels of calcium, sodium, and magnesium and clinical attachment level; however they found no differences in ion levels between smokers and non-smokers. This was also reported by Kolte et al. (19) who found no differences in clinical parameters such as probing pocket depth and CAL in smokers with periodontitis and non-smokers with periodontitis. We are unsure how to explain increased salivary potassium levels in patients with chronic periodontitis when compared to the salivary potassium levels in patients with aggressive periodontitis. It is possible that difference between patients with chronic and aggressive periodontitis could be due to small number of participants in each group i.e. 15 and 20 respectively. Bang et al. (22) reported a positive and statistically significant correlation between the concentration of potassium in the gingival crevicular fluid and the mean pocket depths. The finding of Bang et al. (22) is in contrast with our, as we found no differences between patients with periodontal disease and controls regarding salivary potassium levels.

In conclusion, further studies with a larger sample-size are required to confirm the findings of the present study, with a special emphasis on a particular form of periodontitis.

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