

# Estimation of Salivary Cadmium, Calcium and Manganese Level in Periodontal Disease Patients

Suha T. Abd, B.D.S., M.Sc.<sup>1</sup>, Wasan L. Abdulla B.D.S., M.Sc.<sup>2</sup>, Lubaba A. Samad A. AL-Ameer, B.D.S., M.Sc.<sup>3</sup>, Zainab J. Raheem, B.D.S., M.Sc.<sup>4</sup>, Aseel A. Jabor, B.Sc.<sup>5</sup>

<sup>1,2</sup>Assistant Lecturer. Department of Basic Science, College of Dentistry, University of Baghdad, Iraq

<sup>3,4</sup>Assistant Lecturer. Department of Periodontology, College of Dentistry, University of Baghdad, Iraq

<sup>5</sup>Department of periodontology, College of Dentistry, University of Baghdad, Iraq

**Abstract:** *Background:* Periodontal disease is a common, multi-factorial, chronic inflammatory disease that involves degradation of tissues that support teeth, including alveolar bone. Many salivary biochemical markers was to detect inflammatory changes in its early stages is useful. The objective of this investigation was to estimate the concentrations of the Cadmium (Cd), Calcium (Ca) and Manganese (Mn) in saliva of periodontal disease patients and compare the results with the concentrations of these elements in control group. *Materials and methods:* This study include 24 saliva samples for both groups(chronic periodontitis patients and healthy control).All the three salivary elements (Cadmium, Calcium and Manganese) were determined by Flame Atomic Absorption Spectrophotometer using standardized procedure by air – acetylene. *Results:* The results of this study show highly significant difference between chronic periodontitis patients and healthy control subjects in concentration of all the salivary elements (Cadmium, Calcium and Manganese).There was positive correlation between salivary Ca and Cd in two groups but the correlation between Ca and Mn and the correlation between Cd and Mn was negative correlation. *Conclusion:* there was difference in concentration of salivary concentration of Cd, Ca and Mn between chronic periodontitis patients and control subjects.

**Keywords:** salivary Cadmium, periodontal disease, Salivary Calcium and Manganese

## 1. Introduction

Information that obtained from periodontal diagnostic tools can be used for differential diagnosis, localization of disease, and determine the severity of infection and serve as a base for treatment plan, to assess the effectiveness of periodontal therapy <sup>(1)</sup>.

The conventional periodontal diagnosis methods including measurement of the probing pocket depth, clinical attachment level and gingival recession, by using a graduated probe, can't be affected because of many factors like; experience of clinician, probing force, presence of inflammation or long junctional epithelium. These problems lead to inaccuracies in recording the true pocket depth or the stage of inflammation <sup>(2)</sup>.

So the use of biochemical markers to detect inflammatory changes in its early stages is useful; whereas a measurable change in bone density is required to detect the lesion by using the conventional periodontal diagnosis methods<sup>(3)</sup>.

Periodontal disease is a common, multi-factorial, chronic inflammatory disease that involves degradation of tissues that support teeth, including alveolar bone (jaw bone) <sup>(4)</sup>.

The main cause of periodontal disease is a plaque accumulation on the tooth surface and gingival sulcus; plaque bacteria release lipopolysaccharide and microbial peptides that elicit a host inflammatory response <sup>(5)</sup>.

It is possible that environmental factors, such as exposure to toxic metals like Cadmium, can stimulate the release of inflammatory mediators that lead to periodontal-disease-related tissue destruction<sup>(6)</sup>.

Cadmium may be released from intraoral alloys in dental patients, from metal dental bridge in which a Cd-containing alloy used for soldering or from acrylic-based resin for removable dentures; in which Cd can be used as a pigment <sup>(7)</sup>.

The objective of this investigation was to estimate the concentrations of the Cadmium (Cd), Calcium (Ca) and Manganese (Mn) in saliva of periodontal disease patients and compare the results with the concentrations of these elements in control group.

## 2. Material and Methods

### Subjects

A total of 24 non-smokers, male, systemic health patients diagnosed with periodontal disease were included in this study. They were from attendants seeking periodontal treatment in the Department of Periodontics at Teaching Hospital of Baghdad College of Dentistry. Female were excluded from this study to avoid transient changes that occur in periodontal tissues due to hormonal changes. The patients' age was ranged between (30-50) years. Diagnosis of clinically healthy gingiva (for control group 24 male subjects) & periodontal disease was carried out by a periodontist, based on the criteria of Gingival Index by <sup>(8)</sup> & Clinical Attachment Level (CAL); which can be defined as the distance from the cemento- enamel junction to the location of the inserted probe tip (bottom of gingival crevice or periodontal pocket)<sup>(9)</sup>.The exclusion criteria were including: any patient had history of any systemic disease, taking any medications or mouth wash, alcohol consumption or have mucosal lesions.

### Sample Collection

Five ml of unstimulated saliva was collected from each subject before clinical examination. Each Subject was asked to rinse his mouth with water to insure removal of debris, then waiting for 1-2 min for water clearance. The saliva was collected into small plastic polyethylene tube and the sampling time was early in the morning. The collected saliva was centrifuged at 4000 rpm for 10 minutes. The centrifuged supernatants were stored at (-80°C) until time of analysis.

### Biochemical analysis

The biochemical analysis was done at Poisoning Consultation Center, Medical city. Frozen saliva were allowed to thaw and come to room temperature before their analysis. Thereafter, they were subjected to biochemical analysis. All the three salivary elements (Cadmium, Calcium and Manganese) were determined by Flame Atomic Absorption Spectrophotometer using standardized procedure by air – acetylene.

### Statistical analysis

- Data were analyzed through the use of SPSS (Statistical Process for Social Science), version 14.
- The data are normally distributed according to the Shapiro–Wilk tests (test of normality) ( $p < 0.05$ ).
- Descriptive statistics in the form of mean, standard deviation, bar chart, minimum and maximum were used in this study.
- Inferential statistics in the form of Student t-test, p-value and Pearson correlation were also included. The level of significance was accepted at  $P < 0.05$ , and highly significance when  $P < 0.01$ .

### 3. Results

In this study, the concentrations of the Cadmium, Calcium and Manganese were measured in the saliva of the twenty four patients with periodontal disease group and twenty four subjects with healthy periodontium control group.

The concentration of Ca (which is an essential element) was the highest in the saliva of periodontal disease group (**2.99±0.62 mg/dl**); followed by Cd (**0.18±0.02 µg/dl**) and Mn (**0.04±0.001 µg/dl**). Similarly, in control group, the concentration of Ca (**4.72±0.31mg/dl**) was the highest among other elements, Cd (**0.11±0.03µg/dl**) & Mn (**0.03±0.01µg/dl**).

In table (2), t-test was used to determine the p- value in this study; there were highly significant differences between the concentrations of the Cd, Ca and Mn in the study and control groups.

Table 3 show pearson correlation between the selected elements in saliva of the study and control groups. There was positive correlation between salivary Ca and Cd in two groups but the correlation between Ca and Mn and the correlation between Cd and Mn was negative correlation.

### 4. Discussion

Many studies had related the inorganic minerals in the oral fluid and periodontal disease. Increased level of metals in saliva probably affects the mineralization of dental plaque and hence calculus formation<sup>(10)</sup>. Unremoved dental calculus can cause gingivitis that can further develop into periodontitis<sup>(11)</sup>.

The highest concentration of Ca among other elements in the study group (**2.99±0.62 mg/dl**) was similar to the result obtained by Herman et al., in 2016, but disagree with Basima& Omar in 2012<sup>(10)</sup>; who found in their study the highest concentrations in the study group (chronic periodontitis group) was Na, followed by Mg & Ca. Sew et al<sup>(12)</sup> found higher calcium concentration in periodontitis affected subjects. The oral fluid concentration of Ca can be a risk factor for the development of periodontal diseases.

The highest concentration of Ca in the control group was disagreeing with Basima& Omar<sup>(10)</sup>, where they found a high concentration was Na, followed by Mg & Ca. The concentration of Mn (essential element) (**0.04±0.001 µg/dl**) in the study group was lower than results of Herman et al.<sup>(13)</sup> (**4.11µg/dl**) and Al-Rawi and Talabani<sup>(14)</sup> (**0.6mg/dl**), this differences can be related to the effect of diet on the concentrations of salivary metals<sup>(13)</sup>. The concentration of Cd (a toxic metal) in saliva of study group was (**0.18±0.02 µg/dl**), which is higher than concentration in control group (**0.11±0.03 µg/dl**).

Al-Rawi and Talabani<sup>(14)</sup>, did not found any concentration of Cd in saliva of control group (**0.000 ppm**), while Herman et al.<sup>(13)</sup> found the mean concentration of Cd was higher in control group than study group (periodontal disease group). Exposure to Cadmium cause stimulation of cells to produce prostaglandin, cytokines [such as, interleukins, tumor necrosis factor (TNF-α)], and matrix metalloproteinases (MMP)<sup>(15)</sup>. So, according to this evidence, exposure to Cd leads to exaggeration of periodontal disease.

According to Person correlations, the concentration of Cd increase with the increase of Ca level. Romare and Lundholm<sup>(16)</sup> found the Cd promotes the release of calcium from organ cultures of neonatal mouse calvaria, and this effect is dependent on the induction of the prostaglandin-synthesizing enzyme cyclooxygenase-2.

In the control group, the concentration of Ca increase strongly with decrease in the Mn concentration, this is related to decrease in bacterial pathogenicity, because the Mn<sup>2+</sup> appears to be essential for bacterial virulence factor<sup>(17)</sup>.

### References

- [1] Taba M Jr, Kinney J, Kim AS, Giannobile WV. (2005): Diagnostic Biomarkers for Oral and Periodontal Diseases. Dent Clin North A. 49:551-72.
- [2] Reddy MS. (1997): The use of periodontal probes and radiographs in clinical trials of diagnostic tests. Ann Periodontol. 2: 113-122.

[3] McCauley LK, Nohutcu RM.(2002): Mediators of periodontal osseous destruction and remodeling: Principles and implications for diagnosis and therapy. J Periodontol. 73(11): 1377-91.

[4] Pihlstrom BL, Michalowicz BS, Johnson NW.(2005): Periodontal diseases. Lancet. 366:1809–1820.

[5] Preshaw PM, Seymour RA, Heasman PA.(2004): Current concepts in periodontal pathogenesis. Dent Update. 31:570–578.

[6] Arora M, Weuve J, Schwartz J, Wright R. (2009): Association of Environmental Cadmium Exposure with Periodontal Disease in U.S. Adults. Environmental Health Perspectives, May . 117 (5).

[7] Guzzi Gianpaolo, Paolo D. Pigatto, Anna Ronchi. (2009): Periodontal disease and environmental Cadmium exposure. Environmental Health Perspectives, December .117 (12): 535-536.

[8] Loe H.(1967): the gingival Index, the Plaque Index and retention index system. Journal of Periodontology. 38(6): 610-616.

[9] Carranza FA, Newman MG.(1996):Clinical Periodontology. 8<sup>th</sup> edition.

[10] Ali BG, Ali OH.(2012): Detection of salivary flow rate and minerals in smokers and non-smokers with chronic periodontitis (clinical and biochemical study). J Bagh College Dentistry. 24:68–71.

[11] Salminen A, GURSOY UK, Paju S, Hyvärinen K, Mäntylä P, Buhlin K, Pussinen PJ.(2014): Salivary biomarkers of bacterial burden, inflammatory response, and tissue destruction in periodontitis. J ClinPeriodontol. 41:442–450.

[12] Sewón LA, Karjalainen SM, Söderling E, Lapinleimu H, Simell O.(1998): Association between salivary calcium and oral health. J ClinPeriodontol. 25:915–919.

[13] Herman, M., Golasik, M., Piekoszewski, W. et al.(2016):Essential and Toxic Metals in Oral Fluid—a Potential Role in the Diagnosis of Periodontal Diseases. Biol Trace Elem Res ; published on line.

[14] Al-Rawi NH, Talabani NG.(2005): Quantitative analysis of trace elements in saliva of oral cancer patients from Iraq. J College Dentistry. 17:32–35.

[15] Hemdan NY, Emmrich F, Sack U, Wichmann G, Lehmann J, Adham K, et al.(2006): The in vitro immune modulation by cadmium depends on the way of cell activation. Toxicology. 222:37–45.

[16] Romare A, Lundholm CE.(1999): Cadmium-induced calcium release and prostaglandin E2 production in neonatal mouse calvaria are dependent on cox-2 induction and protein kinase C activation. Arch Toxicol. 73:223–228.

[17] Krisztina M, Papp-Wallace, Michael E. Maguire (2016): Manganese Transport and the Role of Manganese in Virulence. Annual Review of Microbiology, .60: 187-209.

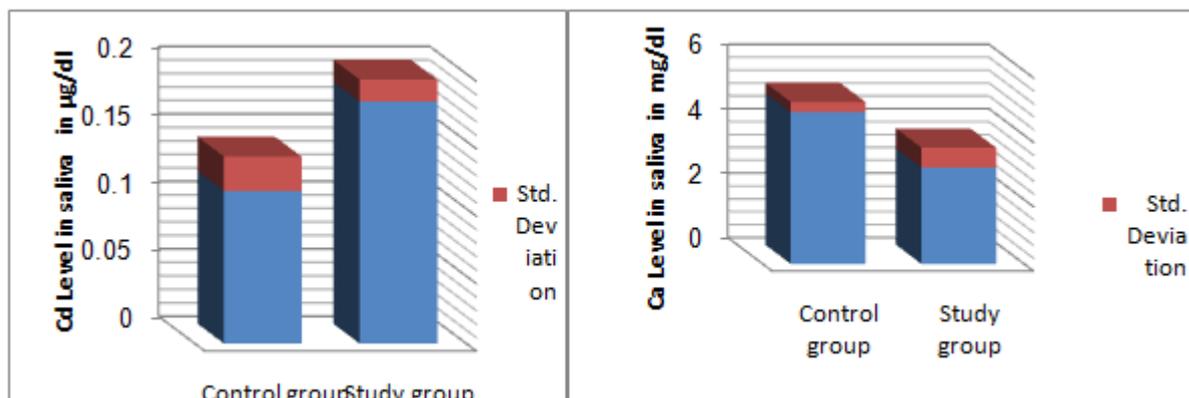
**Table 1:** Descriptive Statistics for the distribution of Cadmium, Calcium and Manganese in the saliva of periodontal disease patients group and healthy periodontium control group

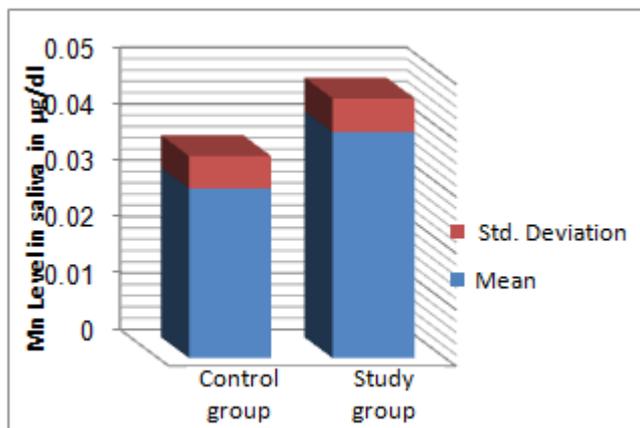
**Tests of Normality**

	Group	Shapiro-Wilk		
		Statistic	df	Sig.
Cd	control	.907	24	.031
	patient	.812	24	.000q
Ca	control	.949	24	.256
	patient	.974	24	.757
Mn	control	.938	24	.147
	patient	.974	24	.756

\* This is a lower bound of the true significance. a Lilliefors Significance Correction.

Elements	Patients/ N= 24				Control / N= 24			
	Min	Max	Mean	±SD	Min	Max	Mean	±SD
Cd (µg/dl)	0.14	0.21	0.18	0.02	0.07	0.15	0.11	0.03
Ca (mg/dl)	1.80	4.10	2.99	0.62	4.20	5.20	4.72	0.31
Mn (µg/dl)	0.03	0.05	0.04	0.001	0.02	0.04	0.03	0.01





**Figure 1:** Mean and standard deviation of Cd, Ca&Mn level in saliva of two groups.

**Table 2:** The significance level for the two groups (study and control) by paired samples t-test.

Salivary elements	Study group mean	Control group mean	t-test	p-value	Sig.
Cd (µg/dl)	0.18	0.11	10.765	0.000	HS
Ca (mg/dl)	2.99	4.72	-12.150	0.000	HS
Mn (µg/dl)	0.04	0.03	5.889	0.000	HS

HS: highly significant

**Table 3:** Pearson correlations between the selected elements in saliva of the study and control groups

	Study group			Control group		
	Cd	Ca	Mn	Cd	Ca	Mn
Cd		0.193	-0.226	Cd	0.331	-0.122
Ca			-0.045	Ca		-0.598**
Mn				Mn		

\*\* Correlation is significant at the 0.01 level (2-tailed).