INFLUENCE OF SMOKING ON THE SALIVARY AND BLOOD CONCENTRATION OF SOME BIVALENT CATIONS IN PATIENTS WITH CHRONIC PERIODONTITIS

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Abstract

The purpose of this study was to determine whether chronic periodontitis can stand behind the modifications observed in the salivary and blood concentration of some bivalent cations (Calcium, Magnesium, Zinc and Copper).

The investigations were performed on an experimental group of 30 patients with clinically-onset chronic periodontitis, and on a control one, including 30 periodontitis-free patients.

Total saliva samples were obtained as “first time in the morning” then weighed and processed. Cations were read on an Atomic Absorption Spectrophotometer (Calcium, Copper and Zinc) and also by Ion Chromatography (Magnesium). The same patients were required to undergo laboratory blood tests for Calcium, Magnesium and Zinc.

The obtained data were normalised, then statistically interpreted using two-tailed heteroscedastic t-Student tests.

The results obtained showed a clear connection of blood magnesium, and also of salivary calcium, magnesium, zinc and copper, to chronic periodontitis.

Salivary cations are therefore related to the local inflammatory status and associated pathological processes. Blood magnesium could be affected by chronic inflammation.

**Keywords**: smoking, bivalent cations, periodontitis.

INTRODUCTION

The meaning of expression “chronic periodontitis” was argued upon for decades. It took a great deal of systematic work [1] to put together all definitions and mechanisms that come under it.

Chronic periodontitis is caused by plaque [2], but disease progression depends on one’s individual susceptibility [3-5]. While many factors have been cited as influencing the progression of the disease, there appears to be a fundamental association with the nature of the host response. Therefore, chronic periodontitis is a chronic inflammatory condition that recognizes the influence of local and general factors in its evolution. Identification of the histological characteristics of the development of chronic periodontitis has also influenced treatment philosophies and how clinicians treat the individual patients affected by it.

Bivalent cations take many involvements in acute and chronic inflammatory pathology. The studied cations (calcium, magnesium, zinc and copper) and many others play complex roles in the human organism. They are found both intracellularly and extracellularly and are crucial for many normal processes within the normal physiology. From this point of view, the tissues of the oral environment have the unique particularity of being in contact with both blood and saliva. Therefore, bivalent cations’ concentration in blood and saliva may have important effects on the physiological processes but also on the pathogenic mechanisms in the oral environment. Salivary concentrations of bivalent cations vary under certain conditions of the oral cavity, head and neck (such as malignant tumours) [6,7].

Under several conditions (glossopyrosis and oropyrosis), salivary magnesium is constantly low [8].

Variations in the concentration of salivary cations are sometimes consistent with, sometimes different from the serum concentrations of the same cations, under different pathological conditions.
In periodontitis, Kuraner [9] identified an increase in serum calcium and a decrease in plasma zinc. In parotid saliva, calcium had a lower concentration than in normal subjects.

The development of gingivitis and, subsequently, of chronic periodontitis lesions, has been classically described as progressing through a series of stages, *i.e.* the initial, early, established and advanced lesions [10]. These stages are not always discernible as distinct entities of their own. The “initial lesion” of chronic periodontitis is a subclinical entity occurring within the first 4 days of plaque accumulation. The characteristic immune response to bacterial enzymes and metabolic end-products is observed as a result of an additional activation of the alternative pathway. There is also a release of tumour necrosis factor-a. However, the presence of an organized plaque biofilm makes neutrophils to release their lysosomal agents, in an act of “abortive phagocytosis”. The action of these extremely active agents exacerbates local tissue damage; however, the lesion is not clinically discernible, and it only occupies 5–10% of the surrounding connective tissues [10]. The resulting “gingivitis” has been well described in humans [11] and in an animal model [13]. Seymour *et al.* [12] outlined the development of a perivascular lymphocyte/macrophage lesion, with T lymphocytes dominating at a CD4:CD8 ratio of 2:1, as confirmed by subsequent observations [14]. The lesion was further noted as identical to that of a delayed-type hypersensitivity reaction [15], [12].

The aim of the present study was to assess the differences in bivalent cation concentration (Calcium, Magnesium, Copper and Zinc) in the blood and saliva of patients in their initial status (with and without chronic periodontitis).

**PATIENTS AND METHODS**

The research was developed on adult patients of both sexes.

The first group was formed of adult subjects of both sexes, non-smokers and free from any pathological conditions of the oral cavity.

The second group consisted of adult subjects of both sexes, smokers and free from any pathological conditions of the oral cavity.

The third group consisted of adult subjects of both sexes, non-smokers and with onset chronic periodontitis.

The fourth group included adult subjects of both sexes, smokers and with onset chronic periodontitis.

The demographic data of the four groups are described in Table 1.

**Table 1. Demographic structure of groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I Control non-smokers</th>
<th>Group II Control smokers</th>
<th>Group III Periodontitis non-smokers</th>
<th>Group IV Periodontitis smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of persons</td>
<td>9</td>
<td>21</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>Age</td>
<td>29±5.66</td>
<td>38±8.29</td>
<td>34.33±8.6</td>
<td>31.75±8.93</td>
</tr>
<tr>
<td>Sex distribution M/F</td>
<td>7/2</td>
<td>18/3</td>
<td>1/1</td>
<td>21/7</td>
</tr>
</tbody>
</table>

All groups were built up of adults of both sexes, without any unusual oral pathology (*e.g.* lithiasis, tumours, conditions related to immune depressions, regional infections), with no feature of general maladies.

Inclusion criteria: Patients included in the experimental groups (III and IV) met the following conditions:

- Feature chronic periodontitis;
- Adults
- Fully compliant to a treatment scheme.

Exclusion criteria:
- Patients with massive metallic dental restorations, that would hinder proper readings for copper and zinc;
- Patients that would not comply with a proper and constant hygiene technique (brushing, flossing);
- Patients possibly called on duty in other places (therefore, impossible to follow);
- Patients with drug-induced gingivitis or with other forms of periodontitis;
- Patients with systemic pathologies, such as *diabetes mellitus*, renal insufficiency, liver cirrhosis, malabsorption syndromes;
- Patients with chronic ethylism;
- Pregnant and breast-feeding patients;
- Patients who used drugs that could bias cation levels (such as diuretics, cardiac tonics);
• Patients who used food supplements containing bivalent cations;
• Patients with any kind of psychosis.

The diagnosis criteria for chronic periodontitis were: true loss of gingival-dental attachment; existence of periodontal pockets deeper than 3 mm; radiological loss of interdental septa (horizontal bone loss) or of lamina dura (vertical bone loss) – all these for at least six dental-periodontal units per arch [16].

As to smoking, the groups were divided as follows:
• The first one consisted of 21 periodontitis-free persons, non-smokers.
• The second group included 9 periodontitis-free persons, of which 6 smoked less than 10 cigarettes a day, and 3 smoked more than 10 cigarettes a day.
• The third group consisted of 2 persons with periodontitis, who never smoked.
• The fourth one consisted of 28 persons with periodontitis, of which 20 smoked more than 10 cigarettes a day, and 8 smoked less than 10 cigarettes a day.

The patients were first subjected to an initial clinical examination and thorough anamnesis, and were required to have their Orthopantomogram (OPG) taken, or to produce a recent one, if available. X-ray analyses were eventually obtained for each patient clinically diagnosed with chronic periodontitis.

Periodontal probing followed. A first-generation calibrated periodontal probe, of the ‘O’-type (University of Michigan), with Williams markings, was used. Each tooth (i.e., a tooth that is not so damaged as to prevent measuring) was subject to six measurements, three on the buccal side and three on the oral side; further on, the measurements made were combined to the chart head, and the patients received a clear and final diagnosis.

Plaque indices were calculated, along with a DMF assessment. To avoid the contribution of mechanical overload to periodontal failure, an “occlusal overload” index, which should not exceed 20% for the patients considered for chronic periodontitis, was designed.

The structure of oral and general pathology, as well as that of the hygiene status and scale deposits, is presented in table 2.

### Table 2. Oral and general pathology, hygiene status and scale deposits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I Control non-smokers</th>
<th>Group II Control smokers</th>
<th>Group III Periodontitis non-smokers</th>
<th>Group IV Periodontitis smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral pathology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New caries</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Improper filling</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Fillings with caries</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Root remains</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>General pathology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic inflammations</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neoplasia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chronic medication</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oral hygiene (OH1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.33 – 3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>1.33 – 2</td>
<td>4</td>
<td>6</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>0 – 1</td>
<td>5</td>
<td>14</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Scale deposits</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td>26</td>
</tr>
</tbody>
</table>

**Saliva sampling**

Patients were instructed to produce a saliva sample in a sterile-sealed single-use polyethylene syringe. They would have to do this the morning of the next appointment, just after waking up, before any hygiene or intake (eating or drinking) [17]. After collecting the saliva sample, patients were asked to bring it to the practice as they would come to the appointment.

The patients were also required to perform a routine blood test, or to bring in any recent tests, if available; the tests included total blood calcium, magnesium and zinc, blood cell count and distribution, PCV, Hb and several other parameters. The blood samples and tests were performed at patient chosen locations.

The saliva samples were tare-weighed in ceramic and platinum crucibles on an analytical scale (producer – Balanţa, Sibiu, Romania), then oven-desiccated at 400ºC for 24 hrs. The crucibles were identified with numbers written with a heat-resistant marker. The residue was taken up with 5 ml nitric acid (5%), then flushed and diluted with distilled water up to 50 ml (working volume for the atomic absorption spectrophotometer [AAS]). The solution obtained was filtered (using filter paper) into sterile dry polypropylene containers labelled according to the original sample ID number, after which
the concentrations of cations were read on an AAS in the Faculty of Pharmacy. For magnesium, determinations were performed in the Analytical Chemistry Department of the Chemistry Faculty – “Al.I. Cuza” University of Iași. Statistical interpretation of data was performed with a two-tailed, heteroscedastic type Student t-test.

As far as dental treatments were concerned, for the third and fourth group, the following situation appeared: patients were found with new onset dental caries (13 in the periodontitis groups, 4 in the control ones), with improper fillings (21 in the periodontitis groups, 7 in the control ones), with caries under fillings (7 in the periodontitis groups, 2 in the control ones), with gangrenes (6 in the periodontitis groups), root remains (18 in the periodontitis groups, 3 in the control ones). In the periodontitis groups, 26 patients featured more than one crown (4 ceramic, 11 metal – acrylic, 3 metal – diacrylic and 7 metallic), while the control group included only 15 persons with crowns or bridgework (5 ceramic, 3 metal – acrylic, and 7 metallic). The cervical margins of the crowns were thoroughly checked, and found inappropriate in 24 patients of the periodontitis groups and in 3 patients of the control one (Table 2).

The study was performed according to the EU norms for clinical studies to the endorsements of the Research Ethics Committee of the “Gr.T. Popa” University of Medicine and Pharmacy, Iași.

RESULTS

Several significant differences, possibly caused by known or alleged phenomena in chronic periodontitis, may be noticed in the saliva samples.

Salivary calcium is lower in non-smoker patients with periodontitis. More precisely, in the control groups, salivary calcium has an average value of 84.24 ± 0.87 mg/L (non-smoker)/ 86.88 ± 3.11 mg/L (smoker) while, in the periodontitis groups, salivary calcium registers an average value of 65.27 ± 14.32 mg/L (non-smoker)/ 80.53 ± 16.24 mg/L, p = 0.0049 for periodontitis, p = 0.035 for periodontitis and smoking (Figure 1).

Blood calcium showed no significant differences (p = 0.5894) (Figure 1).

Salivary magnesium is lower in non-smoker patients with periodontitis. More precisely, in the control groups, salivary magnesium has an average value of 1.88 ± 0.31 mg/L (non-smoker)/ 2.06 ± 0.26 mg/L (smoker) while, in the periodontitis groups, salivary magnesium registers an average value of 1.32 ± 0.18 mg/L (non-smoker)/ 1.56 ± 0.17 mg/L, p = 0.0073 for periodontitis, p = 0.034 for periodontitis and smoking (Figure 2).

Figure 1. Differences in calcium levels

Figure 2. Differences in magnesium levels
On the contrary, salivary magnesium increases in patients with periodontitis. The control group features an average value of \(0.81 \pm 8.5 \times 10^{-3}\) mg/L salivary magnesium while, in periodontal patients, it reaches \(1.07 \pm 0.18\) mg/L, \(p=1.27 \times 10^{-4}\) (Figure 2). Smoking has a significant impact on the salivary level of magnesium in patients who already developed periodontitis. Consequently, group III registered an average salivary magnesium concentration of \(0.82 \pm 0.03\) mg/L, while group IV showed an average value of \(1.12 \pm 0.13\) mg/L (Figure 2).

Blood magnesium decreases in patients with periodontitis, the blood magnesium level registered in control groups I+II being of \(2.05 \pm 0.08\) mg/L, compared to a level of \(1.87 \pm 0.12\) mg/L in patients with periodontitis (groups III and IV), \(p = 0.0049\) (Figure 2). Smoking had no influence on the blood magnesium level (group I registered a value of \(2.03 \pm 0.14\) mg/L, vs group II with \(2.06 \pm 0.16\) mg/L, and group III, respectively, with \(1.93 \pm 0.13\) mg/L vs group IV – with \(1.88 \pm 0.1\) mg/L).

Salivary copper increases in salivary copper levels (healthy average [groups I + II] of \(0.83 \pm 0.18\) mg/L, compared to \(1.2 \pm 0.2\) mg/L in periodontal patients [groups III + IV]), even if not so obviously \((p < 0.05)\) (Figure 3). Smoking has a visible influence on salivary copper concentration, in both healthy (group I had a salivary copper concentration of \(0.66 \pm 0.01\) mg/L vs group II, registering \(0.92 \pm 0.16\) mg/L) and perio patients (group III had a salivary copper concentration of \(0.99 \pm 0.21\) mg/L vs group IV, registering a value of \(1.18 \pm 0.15\) mg/L).

Blood copper was not assessed.

Salivary zinc is not accurately distributed among patients with periodontitis (\(0.9 \pm 0.62\) mg/L), but it is definitely low in healthy subjects (\(0.27 \pm 0.13\) mg/L). The difference is statistically significant \((p = 0.0014)\) (Figure 4). Smoking does not only induce differences, but also increases the individual variability of salivary concentration. In healthy patients, the average level decreases with smoking (group I recording a salivary zinc level of \(0.38 \pm 0.01\) mg/L, vs group II – with \(0.21 \pm 0.12\) mg/L), while in perio patients it increases with smoking (group III had a salivary zinc concentration of \(0.49 \pm 0.38\) mg/L vs group IV – with \(0.94 \pm 0.65\) mg/L) (Figure 4).
a broad interval that mere average and standard deviation were not sufficient to produce clustering by periodontitis and smoking.

**DISCUSSION**

Bivalent cations have various involvements in acute and chronic inflammatory mechanisms. Extracellular calcium triggers NLRP3 inflammation, which enables inflammatory monocyte and macrophage to release high levels of interleukin 1β [18]. Some elements of the molecular mechanism of inflammation depend on the calcium release from intracellular structures, or on the entrance of calcium ions from the extracellular area into the cells [19]. Human macrophages’ activity is calcium-dependent [20].

Lowering of salivary calcium may be charged to the scale deposition occurring through precipitation of soluble calcium from the saliva, therefore decreasing calcium concentration. This mechanism could also explain the relative uniformity of figures in the experimental group (σ = 7.65, that is 14.06%) compared to the control one (σ = 34.3, that is 29.7%), probably by reaching a saturation threshold of the precipitation phenomenon.

Magnesium is an essential bivalent metal for the activity of over 300 enzymes. It features an anti-oxidant activity since it reduces the formation of peroxide radicals. It is involved in the modulation of vascular permeability and in the endothelial function. Magnesium reduces the synthesis of cytokines [21]. Administration of magnesium suppressed some inflammatory responses in various tissues [22].

In rats with experimental magnesium deficiency, an inflammatory syndrome was discovered, characterized by the increase of the release of inflammatory cytokines, and by leukocyte activation. The synthesis of acute phase proteins increases [23].

The relative uniformity of the salivary magnesium values in the control group is probably caused by a strict tuning of salivary secretion in periodontitis.

In the present research, the serum level of calcium and magnesium does not feature significant differences in patients with chronic periodontitis (either smoker or non-smoker), comparatively with the control groups.

Calcium salivary concentration is significantly lower in patients with periodontitis. Smoking did not affect the level of salivary calcium. Smoking did not affect the level of salivary calcium in either the periodontitis or control group.

Magnesium salivary concentration is significantly higher in the periodontitis group, comparatively with the control one, and higher in smokers suffering from periodontitis than in non-smokers also affected with periodontitis. Thus, when the salivary Magnesium/salivary Calcium ratio is obviously higher in patients with this periodontal condition, magnesium is suspected to reduce the bacterial LPS-induced inflammation [24], an increased salivary magnesium level possibly contributing to the put-off of periodontal inflammation, mostly caused by bacteria. Accordingly, a reduced level of magnesium increases phagocytosis and the yield of reactive oxygen species in neutrophils [25].

An increased magnesium level could diminish apoptosis and also sustain tissue regeneration [26]. A higher salivary magnesium level could have a reducing action on the destruction of some pro-inflammatory cytokines, such as TNF-α and IL-6 [27]. Magnesium deficiency supports the development of inflammatory conditions [28].

The increase of copper, not very obvious, can be charged to other causes, such as contamination from the present or prior bronze bridgework and/or smoking (Figure 4).

Zinc is an essential element for the activity of the immune system. In cases of zinc deficiency, the oxidative stress exacerbates [29]. Administration of zinc diminishes the oxidative stress, inhibiting the development of certain inflammatory processes [30]. The ratio of copper/zinc concentrations is important for the intensity of the oxidative stress. Thus, an increased copper/zinc ratio is correlated with an increased level of the oxidative stress [31]. No data are available on what an increased zinc level could mean. Possibly, this is due to a very host-dependent immune mechanism involving salivary IgA and a complement, as a reaction to the presence and multiplication of periodontal bacteria.

The salivary levels of copper and zinc are significantly increased in patients with chronic periodontitis, compared to their respective control...
groups. The strong increase in the salivary zinc level in patients with periodontitis has no connection with smoking since, in the control group, no significant difference could be observed in salivary zinc concentration among smokers and non-smokers.

The existing data on the variation of salivary and blood concentrations of the above-mentioned bivalent cations in relation to chronic periodontitis are still contradictory. By analyzing the salivary concentrations of calcium in patients (adult smokers and non-smokers) with both chronic and aggressive periodontitis, Kiss [32] found out that the level of salivary calcium in smokers is significantly higher than in non-smoker perio patients (57.76 ± 18.8 mg/L vs 44.6 ± 7.8 mg/L). The higher calcium level was associated to increased bone loss and probing pocket depth.

By analyzing salivary calcium concentration in patients with periodontitis, smokers and non-smokers, Sutei [33] evidenced no significant differences.

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