RESEARCH ON PLASMA AND SALIVA LEVELS OF SOME BIVALENT CATIONS IN PATIENTS WITH CHRONIC PERIODONTITIS
(SALIVARY CATIONS IN CHRONIC PERIODONTITIS)

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RESEARCH ON PLASMA AND SALIVA LEVELS OF SOME BIVALENT CATIONS IN PATIENTS WITH CHRONIC PERIODONTITIS (Abstract): The purpose of this study was to determine whether chronic periodontitis can stand behind modifications in the salivary and blood concentration of some bivalent cations (Calcium, Magnesium, Zinc and Copper). For this purpose, we formed a group of 30 adult patients with clinically onset chronic periodontitis, and another one of 30 healthy patients as control. Both groups were free from acute oral pathology and general illnesses. The groups were divided again according to the habit of smoking. Total saliva samples were obtained as “first time in the morning”, then weighed and processed. Cations were read on Atomic Absorption Spectrophotometer and by Ion Chromatography (Magnesium). The same patients were required to undergo laboratory blood tests for Calcium, Magnesium and Zinc. Data obtained was normalised, then statistically interpreted using two-tailed heteroscedastic t-Student tests. Our data confirmed the existence of a connection between salivary calcium, magnesium, zinc and copper, and of blood magnesium, and chronic periodontitis. Salivary calcium and magnesium are affected by smoking.

Keywords: CHRONIC PERIODONTITIS, CALCIUM, MAGNESIUM, ZINC, COPPER.

The meaning of the words “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 an individual’s susceptibility (3, 4, 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 individual patients affected by chronic periodontitis.

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 intracellular and extracellular 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 to be in contact with both blood and saliva. Therefore, bivalent cations’ concentration in blood and saliva may have important effects on physiological processes but also on pathogenic mechanisms in the oral environment. Salivary concentrations of bivalent cations vary in certain conditions of the oral cavity and of the head and neck (such as malignant tumours) (6, 7, 8).

There are several conditions (glossopyrosis and oropyrosis) where salivary magnesium is constantly low (9).

Variations in the concentration of salivary cations are sometimes consistent with and some other times different than the serum concentrations of the same cations, under different pathological conditions.

The development of gingivitis and subsequently of the chronic periodontitis lesion 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 in their own right. The “initial lesion” of chronic periodontitis is a subclinical entity occurring within the first four days of plaque accumulation. The characteristic immune response to bacterial enzymes and metabolic end products is observed as a result of complement activation of the alternative pathway. There is also a release of tumor necrosis factor-a. However, the presence of an organized plaque biofilm induces the 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 only occupies 5–10% of the surrounding connective tissues (10). A number of other factors have been described as being involved in the pathogenesis of chronic periodontitis (11, 12, 13, 14, 15).

The aim of this study was to assess the differences in some 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 aimed at adult patients of both sexes.

The first lot consisted of adult subjects of both sexes, non-smokers and free from any pathological conditions of the oral cavity. The second lot consisted of adult subjects of both sexes, smokers and free from acute pathological conditions of the oral cavity. The third lot consisted of adult subjects of both sexes, non-smokers and with onset chronic periodontitis. The fourth lot consisted of adult subjects of both sexes, smokers and with onset chronic periodontitis. The demographic data of the four lots are described in Table I.

**TABLE I**

Demographic structure of the lots

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lot I Control non-smokers</th>
<th>Lot II Control smokers</th>
<th>Lot III Periodontitis non-smokers</th>
<th>Lot IV Periodontitis smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of persons</td>
<td>9</td>
<td>21</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>Age &amp; Sex</td>
<td></td>
<td></td>
<td></td>
<td></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>11/1</td>
<td>11/7</td>
</tr>
</tbody>
</table>
Research on plasma and saliva levels of some bivalent cations in patients with chronic periodontitis (Salivary cations in chronic periodontitis)

Lots I and II were built up of adults of both sexes, without acute oral pathology, and who did not feature general illnesses.

Inclusion criteria: Patients included in the study lots needed to:
- be of age;
- feature chronic periodontitis;
- be 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 assuming a proper and constant hygiene technique (brushing, flossing);
- Patients that were afterwards called on duty in other places (therefore, impossible to follow);
- Patients with drug-induced gingivitis or with other forms of periodontitis;
- Patients with chronic ethylism;
- Pregnant and breast-feeding patients;
- Patients with systemic illnesses such as diabetes mellitus, renal insufficiency, liver cirrhosis, malabsorption syndromes;
- Patients who used food supplements that contained bivalent cations;
- Patients with any kind of psychosis.
- Patients who used drugs that could bias cation levels (such as diuretics, cardiac tonics).

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).

In what smoking was concerned, the lots were divided as follows:
- The first lot consisted of 21 periodontitis-free persons, non-smokers.
- The second lot consisted of 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 lot consisted of 12 persons with periodontitis who didn’t smoke at all.
- The fourth lot consisted of 18 persons with periodontitis, of which 10 smoked more than 10 cigarettes a day, and 8 smoked less than 10 cigarettes a day.

Patients were first subjected to an initial clinical examination and a thorough anamnesis.

The patients were required to have their Orthopantomogram (OPG) taken, or to produce a recent one if available. The x-rays were eventually obtained for each patient clinically diagnosed with chronic periodontitis.

Periodontal probing followed. We used a first-generation calibrated periodontal probe, of the ‘O’-type by the University of Michigan, with Williams’s markings. Each tooth (meaning 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. After performing such measurements, and combining them to the chart head, patients received a clear and final diagnosis.

The structure of oral and general pathology, as well as that of hygiene status and scale deposits may be found in table II.

Saliva sampling
Patients were then 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 hy-
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 (Balanța, Sibiu, Romania), then oven-desiccated at 400°C for 24 hours. The crucibles were identified with numbers written with 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. Containers were labeled according to the original sample ID number and then the concentrations of cations were read on an AAS in the Faculty of Pharmacy. For magnesium, the determinations were performed by the Analytical Chemistry department of the Chemistry Faculty in the “Al. I. Cuza” University Iași.

Statistic interpretation of the data series was performed using a t-Student test, two-tailed and of the heteroscedastic type.

### TABLE II

**Oral and general pathology, hygiene status and scale deposits**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lot I Control non-smokers</th>
<th>Lot II Control smokers</th>
<th>Lot III Periodontitis non-smokers</th>
<th>Lot IV Periodontitis smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oral pathology</strong></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>Gangrenes</td>
<td>0</td>
<td>0</td>
<td>1</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><strong>General pathology</strong></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><strong>Oral hygiene (OHI)</strong></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>12</td>
<td>16</td>
</tr>
</tbody>
</table>

As far as dental treatments were concerned, for the third and fourth lot, we found the following situation: Patients were found with new onset dental caries (13 in the periodontitis lots, 4 in the control lots), with improper fillings (21 in the periodontitis...
Research on plasma and saliva levels of some bivalent cations in patients with chronic periodontitis (Salivary cations in chronic periodontitis)

In saliva samples, there were several significant differences. We tried to allocate such differences to known or alleged phenomena in chronic periodontitis.

Salivary calcium was lower in non-smoker patients with periodontitis. That is, in control groups salivary calcium had an average of 84.24±0.87 mg/L (lot I) / 86.88±3.11 mg/L (lot II), while in the periodontitis lots, salivary calcium had an average of 53.37±6.57 mg/L (lot III) / 56.12±8.45 mg/L (lot IV), p<0.05 for periodontitis, p<0.05 for periodontitis and smoking (fig. 1).

Blood calcium did not feature any significant differences (p=0.5894) (Fig. 1).

Contrary, salivary magnesium increased in patients with periodontitis. The control group featured an average of 0.81 ± 8.5x10⁻³ mg/L salivary magnesium, while in periodontal patients, it reached 1.07 ± 0.18 mg/L, p<0.05 (fig. 2).

Blood magnesium decreased in patients with periodontitis. We found in the control group an average blood magnesium of 2.05 ± 0.08 mg/L, compared to an average of 1.87 ± 0.12 mg/L in patients with periodontitis, p<0.05 (fig. 2).

**RESULTS**

In saliva samples, there were several significant differences. We tried to allocate such differences to known or alleged phenomena in chronic periodontitis.
Fig. 2. Differences in magnesium levels

Smoking severely affected the salivary magnesium level in patients with periodontitis. Lot III had 0.82±0.03 mg/L, while lot IV had 1.12±0.13 mg/L, p<0.05. Salivary magnesium was also affected by smoking in healthy patients, lot I having 0.72±0.03 mg/L and lot II having 0.84±0.02 mg/L, but the statistical significance was not enough to state a clear difference, p=0.05.

Salivary copper increased in periodontitis (healthy average of 0.83 ± 0.18 mg/L, compared to 1.2 ± 0.2 mg/L in the periodontal patients), but this was not so evident (p<0.05) (fig. 3). Smoking had a positive effect on salivary copper in healthy subjects (lot I having a “basal” 0.66±0.01 mg/L while lot II had 0.92±0.16 mg/L, p<0.05) as well as in those with chronic periodontitis (lot III had 0.99±0.21 mg/L while lot IV had 1.18±0.15 mg/L, p<0.05).

Fig. 3. Differences in salivary copper levels

Salivary zinc was not that accurately distributed among patients with periodontitis (lot III with 0.37 ± 0.01 mg/L, and lot IV with 0.21 ± 0.12 mg/L), but was defi-
nitely higher in healthy subjects (lot I with 0.48 ± 0.05 mg/L and lot II with 0.53 ± 0.18 mg/L). The difference was statistically significant (p<0.05) (fig.4).

**DISCUSSION**

Bivalent cations have various involvements in acute and chronic inflammatory mechanisms. Extracellular calcium triggers NLRP3 inflammasome 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).

In periodontitis, Kuraner (21) identified an increase in serum calcium and a diminishment of plasma zinc. In parotid saliva, calcium had a lower concentration than in normal subjects.

Lowering of salivary calcium may be charged to the deposition of scale that occurs by precipitation of soluble calcium from the saliva, therefore decreasing calcium concentration. This mechanism could also explain the relative uniformity of figures in the working lot (σ=7.65, that is 14.06%) compared to the control lot (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 forming of peroxide radicals. It is involved in the modulation of vascular permeability, and in the endothelial function. Magnesium reduces the synthesis of cytokines (22). Administration of magnesium suppressed some inflammatory responses in various tissues (23).

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 (24).

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

In our research the serum level of calcium and magnesium does not feature significant differences in patients with chronic periodontitis (either smoker or non-smoker)
compared to the control lots.

The salivary concentration of calcium is significantly lower though in patients with periodontitis. Smoking did not affect, either in the periodontitis lot or in the control lot, the level of salivary calcium.

The salivary concentration of magnesium is significantly higher in the periodontitis lot compared to the control lot, and higher in the smokers with periodontitis than in the non-smokers, also with periodontitis. Thus, in the case where 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 (25), and an increased salivary magnesium level could contribute to the put-off of the periodontal inflammation, mostly caused by bacteria. Respectively, a reduced level of magnesium increases phagocytosis and the yield of reactive oxygen species in the neutrophils (26).

An increased magnesium level could diminish apoptosis and also sustain tissue regeneration (27). A higher salivary magnesium level could have a diminishing action on the destruction of some pro-inflammatory cytokines such as TNF-α and IL-6 (22). Magnesium deficiency supports the development of inflammatory conditions (28).

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

Zinc is an essential element for the activity of the immune system. In case of zinc deficiency, the oxidative stress exacerbates (29). Administration of zinc diminishes the oxidative stress hence 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 increase of the copper / zinc ratio is correlated to an increase of the level of the oxidative stress (31). There is no assertion of what an increased zinc level could mean. Probably it is due to a very host-dependent immune mechanism involving salivary IgA and complement, as a reaction to the presence and multiplication of periodontal bacteria.

The salivary level of copper is significantly increased in patients with chronic periodontitis, compared to their respective control lot. The accentuated decrease in the salivary zinc level in patients with periodontitis has an alleged connection with smoking, since in the control lot there is no significant difference in salivary zinc concentration between 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 with periodontitis (both chronic and aggressive periodontitis), adult smoker and non-smoker, Kiss (32) found that the level of salivary calcium in smokers is significantly higher than in non-smoker periodontitis patients (57.76 ± 18.8 mg/L vs. 44.6 ± 7.8 mg/L). The higher calcium level was associated to an increased bone loss and probing pocket depth.

By analyzing salivary calcium concentration in patients with periodontitis, smokers and non-smokers, Sutei (33) found no significant differences.

Kolte and his team analyzed in 2012 the saliva in smokers compared to non-smokers and found that total proteins, calcium, magnesium, and zinc are significantly reduced in smokers vs. non-smokers (34).
The same diminishment of calcium and magnesium concentration in the smokers’ saliva was also found by Zuabi et al (35).

CONCLUSIONS
In our research we were unable to depict significant differences in salivary calcium from patients with chronic periodontitis, that is in smokers vs. non-smokers, but in both cases these concentrations were significantly lower than the concentrations in the saliva of healthy subjects, both smokers and non-smokers, but in both situations these concentrations were significantly lower than those in the saliva of periodontally healthy patients, either smoker or non-smoker.

In our study, the level of salivary magnesium in smokers with chronic periodontitis is significantly higher compared to both healthy smokers and non-smokers with chronic periodontitis. The heterogeneity of the data in the literature is rather difficult to explain. Our results show a clear increase of the salivary copper / zinc ratio and the diminishment of salivary and blood zinc in patients with chronic periodontitis, compared to the healthy ones. We reckon that the lower zinc level may cause a slower and weaker immune activity, thus supporting the development of bacteria involved in the pathogenicity of chronic periodontitis.

Thus, testing the salivary concentrations of bivalent cations may be useful for the assessment of patients with periodontitis, smokers or non-smokers.

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**RECEPTOR-MEDIATED RECOGNITION OF MYCOBACTERIAL PATHOGENS**

*Mycobacterium* is a genus of Actinobacteria that includes more than 50 different species, ranging in virulence from the non-pathogenic *M. smegmatis* to the causative agent of tuberculosis (TB) in humans, *M. tuberculosis*. Infection with mycobacteria most commonly occurs through inhalation or ingestion of bacilli. The bacilli are phagocytosed by host macrophages and dendritic cells (DCs) at the site of infection, for example by alveolar macrophages in *M. tuberculosis*-infected lungs or by intestinal macrophages in animals infected with *M. avium* subsp. *paratuberculosis* (MAP). Mycobacteria have evolved a range of mechanisms to circumvent phagosome maturation, preventing lysosomal degradation, and are therefore able to both survive and replicate inside the host phagosome. In order for mycobacteria to be phagocytosed by macrophages and DCs, the pathogen is first recognized by pattern recognition receptors (PRRs) on the host cell. This is achieved through the recognition of highly conserved molecular structures found on the surface of pathogens, often critical for microbial survival, termed *pathogen associated molecular patterns* (PAMPs). Several families of PRRs exist, all of which are capable of recognizing a different repertoire of PAMPs; including plasma membrane-bound and intracellular Toll-like receptors (TLRs) and cytosolic NOD-like receptors (NLRs) and RIG-I like receptors (RLRs). Future studies targeting different combinations of receptors may lead to a better appreciation of the complex interactions between phagocyte and mycobacterium. Ultimately, increasing our understanding of innate immune recognition of mycobacterial pathogens will aid in our ability to treat and prevent mycobacterial diseases. (Kate E. Killick, Clíona Ní Cheallaigh, Clíona O’Farrelly, et al. Receptor-mediated recognition of mycobacterial pathogens. *Cellular Microbiology* (2013) 15(9): 1484–1495)

*Doina Butcovan*