Immune markers in oral discomfort patients before and after elimination of oral galvanism

Stepan Podzimek, Milan Tomka, Pavla Sommerova, Yelena Lyuya-Mi, Jirina Bartova, Jarmila Prochazkova

Institute of Clinical and Experimental Dental Medicine, General University Hospital and First Faculty of Medicine, Charles University, Prague, Czech Republic

Correspondence to: Stepan Podzimek, PhD.
Institute of Clinical and Experimental Dental Medicine, General University Hospital and First Faculty of Medicine, Charles University Karlovo namesti 32, 120 00 Praha 2, Czech Republic.
tel: +420 224966801; e-mail: podzimek@vus.cz

Submitted: 2013-03-12 Accepted: 2013-05-15 Published online: 2014-01-15

Key words: oral discomfort; oral galvanism; electro active restorations removal; IgA1; IgA2; sIgA; lysozyme; antiIgA/HSP60

Abstract

BACKGROUND: An enhanced release of metals in the mouth due to galvanic cell formation is considered to be one of the causes of oral discomfort. The aim of this study was to investigate the influence of galvanic cell on salivary immune defense factors.

MATERIAL AND METHODS: The levels of IgA1, IgA2, sIgA, lysozyme and antiIgA/HSP60 were evaluated in representative samples from 159 patients with galvanism, from 177 patients without galvanism and in two control groups. All the participants underwent personal history taking, clinical examination, galvanic currents measurement and saliva collection.

RESULTS: Electro active dental materials were removed in 30 patients. There was a significant increase in IgA2 level, a significant decrease in antiIgA/HSP60 levels and an increase in IgA1, sIgA and lysozyme levels found after the removal of electro active restorations. Morphological symptoms disappeared in 70% of the treated patients.

CONCLUSION: The study confirmed that pathologic galvanic phenomena influences the immune defense reactions in the oral cavity and thus may cause the symptoms of oral discomfort. A measurement of the galvanism and a subsequent removal of electro active restorations should become a common therapeutic procedure in the patients with oral discomfort.

INTRODUCTION

In the case of patients with oral discomfort an unusual character and intensity of patient’s complaints combined with negative or very discrete and uncommon findings in the oral cavity often lead to diagnostic as well as therapeutic confusion. Dental materials are in a long-term contact with soft and hard tissues of the oral cavity. Due to specific environmental characteristics of the mouth, metal alloys undergo mechanical and electrochemical changes. These changes may contribute to a development of oral discomfort symptoms (Wataha 2000).

The presence of diverse metallic systems in the oral cavity may induce the development and manifestation of electrochemical corrosion, where various dental alloys function as electrodes and fluids in the oral cavity (saliva, crevicular fluid or dietary fluids) as an electrolyte (Holland 1980; Arvidson
& Johansson 1985; Yontchev et al. 1989; Walker et al. 2003; Al-Ali et al. 2005; Ciszewski et al. 2007; Koh et al. 2008; Sutow et al. 2008). An action of the galvanic current may also manifest itself as morphologic changes of oral tissues. Consequently inflammation of the oral mucosa and tongue, paraesthesia, glossodynia, stomatodynia, hyperaemia of the pulp or neuralgia may occur (Kucerova et al. 2002; Sutow et al. 2004). Galvanic effects can be evaluated by measuring galvanic potentials and currents (values over 5 microamperes are considered pathological) (Nilner 1981; Nilner et al. 1982; Axell et al. 1983; Hampf et al. 1987; Kobayashi 1989; Nogi 1989; Kucerova et al. 2002; Wataha 2002; Sutow et al. 2004; Prochazkova et al. 2006; Podzimek et al. 2008) and, in addition, it is relatively easy to eliminate galvanism from the oral cavity by removing electro active dental materials and replacing them by non-metallic ones (Lindh et al. 2002; Prochazkova et al. 2004; Prochazkova et al. 2006).

Local toxic activity of metal ions released from dental metal materials to adjacent tissues may lead to the development of inflammation, activation of immunocompetent cells, production of proinflammatory factors, worsened elimination of metabolic products and, therefore, to the worsening of inflammation (Syrrjanen et al. 1985; Bumgardner & Johansson 1996). In patients with already developed oral cavity inflammatory disease (periodontitis), especially in special subgroup of these patients (pregnant women), the inflammation can have serious consequences (pre-eclampsia) (Straka et al. 2011). The inflammation may become chronic if the causal factor is not removed and an appropriate treatment is not provided. Components of the saliva, such as secretory IgA, IgA1, IgA2 and lysozyme, have a critical role in the mucosal defence against galvanic phenomena (Kerr 1990; Nagler et al. 2002).

The objective of this study was to investigate the influence of pathologic galvanic currents and therefore enhanced release of metal ions from oral metal restorations on immune defense factors of the saliva in patients with oral discomfort.

**MATERIAL AND METHODS**

Study participants from all four groups underwent personal history taking, clinical examination, galvanic currents measurement and saliva collection for establishment of IgA1, IgA2, sIgA, lysozyme and IgA antibodies against heat shock protein 60 (antiIgA/HSP60) levels. Electro active dental materials were removed in 30 patients. For the purposes of this project 397 patients – 91 men and 306 women of an average age of 51 years – were examined.

**Patients with oral discomfort**

There were 336 patients referred for an examination for suspicious “galvanic problems” (inflammatory or lichenoid efflorescences on the mucosa after dental treatment, metallic pigmentation of the gingiva, prosthetic restorations staining and corrosion, tongue burning, burning and shedding of lips and oral mucosa, sour, bitter, salty sensations, metallic taste) or “oral discomfort of an unknown etiology”. Based on the measurement of galvanic currents, these patients were divided into two groups: in 159 patients (34 men and 125 women, an average age of 53.5 years) the pathological values of galvanic currents ranging from 5–50μA were found and these patients constituted the Group DIS+GAL+. Thirty patients from Group DIS+GAL+ had their electro active fillings replaced.

The Group DIS+GAL– consisted of 177 patients (37 men and 140 women, an average age of 52.5 years) with oral discomfort, with no pathological values of galvanic currents found.

**Control groups**

Two control groups were established:

The Group DIS–GAL– consisted of 40 participants (8 men and 32 women, an average age of 49.5 years) without any pathological values of galvanic currents and without any oral discomfort. Dental materials found in the oral cavities of these participants were similar to those used in patients in the Group DIS+GAL+ and in the Group DIS+GAL–.

The Group DIS–GAL–MET– consisted of 21 healthy participants (12 men and 9 women, an average age of 32.5 years) without any pathological values of galvanic currents, without any oral discomfort and without any dental metal fillings or other metallic restoration in their oral cavity.

**Personal history**

All examined persons had a detailed personal history, which focused on the exposure to metals, taken.

**Clinical examination**

All tested persons underwent a detailed examination of the oral tissues including a panoramic X-ray examination. Their examination focused on the identification of dental metal restorations and morphologic signs of oral discomfort – inflammatory or lichenoid changes of the mucosa, tongue or gingiva. The presence of the metal staining of their oral tissues was also evaluated.

**Galvanic currents measurement**

The galvanic currents between dental alloys and gum, tongue, lips or buccal mucosa and between different dental alloys were measured using the Odontologic 2000 device (Embitron; Prague, Czech Republic) (Kucerova et al. 2002). A threshold for galvanic current pathological values (over 5 μA) was established in previous studies (Kucerova et al. 2002; Prochazkova et al. 2006; Podzimek et al. 2008) based on experimental data and previous publications (Holland 1980; Nilner 1981; Nilner et al. 1982; Axell et al. 1983; Arvidson & Johansson 1985; Hampf et al. 1987; Nogi 1989; Walker et al. 2003; Sutow et al. 2004; Sutow et al. 2008).
Non-stimulated saliva (1 ml) from all examined participants was collected and frozen at –18°C. After defrosting the samples a radial immunodiffusion method was used to determine immunologic marker (IgA1, IgA2, secretory IgA and lysozyme) levels. Commercial kits Human IgA Subclass NL BINDARID™ Combi Kit, Human Secretory IgA RID Kit (The Binding Site Ltd.; Birmingham, UK) and Human Lysozyme NL NANORID™ Kit (The Binding Site Ltd.) were used.

The ELISA method (Lequin 2005) was used to measure IgA antibodies against HSP 60 in the saliva.

The measurement was performed in all the groups in randomly selected patients (Group DIS+GAL+ – 41 samples, Group DIS+GAL– – 44 samples, Group DIS–GAL– – 18 samples and Group DIS–GAL–MET– – 15 samples).

**Removal of electro active dental restorations from oral cavity**

Electro active dental restorations were removed from the oral cavity in 30 patients of the Group DIS+GAL+, with morphological symptoms in their oral cavity. Electro active restorations were replaced under the shield of strong antioxidants – vitamin C and selenium.

Special precautions were used to eliminate a contact of the patients with released metal ions from the removed electro active metal restorations (Lindh et al. 2002; Prochazkova et al. 2004).

**Statistical analyses**

Statistical data analysis was performed using the single sample Student’s t-test after logarithmic transformation \( y = \log_{10}(x+1) \) for the quantitative data. Qualitative characteristics of the examined samples were compared using the Pearson test \( \chi^2 \). An analysis was done to determine required number of randomly selected samples from participants from each group in order to determine if statistical differences exists between groups.

All the participants of this study were examined and treated after providing an informed consent statement, with approval of institutional scientific and ethics committees and in accordance with the Helsinki Declaration.

**RESULTS**

**Results of personal history examination**

An unfavorable reaction to metals was subjectively perceived by all patients with oral discomfort (the Group DIS+GAL+ and the Group DIS+GAL–), by one woman from the Group DIS–GAL– (3%) and by no participant from the Group DIS–GAL–MET–. Diverse allergies were found in one half of the patients with oral discomfort (80 participants – the Group DIS+GAL+), in at least one third of patients from the Group DIS+GAL– (49 participants – 28%), in 7 patients from the Group DIS+GAL+ (18%) and in one patient from the Group DIS–GAL–MET– (5%).

**Results of the oral cavity clinical examination**

Clinical signs of inflammatory affections, lichenoid changes or metallic pigmentation were found most frequently on the mucosa and gingiva of the patients from the Group DIS+GAL+ (40%). In the Group DIS+GAL–, a significantly lower number of such affections (16%) was found. In the Group DIS–GAL– only two persons with metallic pigmentation (5%) were found and in the Group DIS–GAL–MET– no such affections were found, as expected. A statistic analysis did not reveal any significant differences in dental metal restorations within the tested Groups DIS+GAL+, DIS+GAL– and DIS–GAL–.

**Galvanic currents measurement**

Among those 397 examined persons pathologic values of galvanic currents were found in 159 patients (Group DIS+GAL+). The mean value of the galvanic current was 13.4±10.3μA. In the remaining three groups, galvanic current values did not reach the limit of 5μA.

When comparing galvanic current maximum values with clinical findings in the patients from the Group DIS+GAL+, we found significantly \( p=0.00004 \) higher values of galvanic currents in the patients with morphological changes on the oral mucosa, tongue and gingiva as compared to the patients without morphological affections. The highest values of galvanic currents were found in the patients with inflammatory and lichenoid changes of the oral mucosa and tongue (22.8±12.5μA; \( p=0.0001 \) compared to the patients without the affections; \( p=0.0118 \) compared to the patients with pigmentation), lower values in the patients with metallic pigmentation (14.0±9.6μA; \( p=0.01016 \) compared to the patients without the affections) and the lowest values in patients with oral discomfort, but without any morphological changes in their oral cavity (1.8±1.3μA).

**Immunologic markers determination and comparison**

**IgA1 levels**

The highest mean concentration of IgA1 in the saliva was found in the Group DIS–GAL–MET– (5506 mg/l) and the lowest in the Group DIS+GAL+ (1154 mg/l). Statistically significant differences were found between the Group DIS+GAL+ and the Group DIS+GAL– \( p=0.036 \), the Group DIS+GAL+ and the Group DIS–GAL–MET– \( p=0.004 \) and between the Group DIS+GAL– and the Group DIS–GAL–MET– \( p=0.012 \) (Table 1).

**IgA2 levels**

The highest mean concentration of IgA2 in the saliva was observed in the Group DIS–GAL–MET– (778 mg/l)
Immune markers in oral discomfort patients

The highest mean concentration of secretory IgA was found in the Group DIS–GAL– (669 mg/l) and the lowest in the Group DIS+GAL+ (266 mg/l). Statistically significant differences were discovered between the Group DIS+GAL+ and the Group DIS–GAL– (p=0.001), the Group DIS+GAL+ and the Group DIS–GAL–MET– (p=0.02), and the Group DIS+GAL+ and the Group DIS–GAL–MET– (p=0.049) (Table 3).

Lysozyme levels

The highest value of lysozyme mean concentration was found in the Group DIS–GAL– (6 mg/l) and the lowest in the Group DIS+GAL+ (1.1 mg/l). Statistically significant differences were discovered between the Group DIS+GAL+ and the Group DIS–GAL– (p=0.001), the Group DIS+GAL+ and the Group DIS–GAL–MET– (p=0.002) (Table 4).

Levels of IgA antibodies against HSP 60

The highest mean concentration of IgA antibodies against HSP 60 was found in the Group DIS+GAL+ and the lowest in the Group DIS+GAL–GAL+ (309 mg/l). Differences between the mean levels in the Group DIS+GAL+ and the Group DIS+GAL– (p=0.018), the Group DIS+GAL+ and the Group DIS–GAL– (p=0.004), the Group DIS+GAL+ and the Group DIS–GAL–MET– (p=0.001) were statistically significant (Table 2).

Secretory IgA levels

The highest mean concentration of secretory IgA was found in the Group DIS–GAL– (669 mg/l) and the lowest in the Group DIS+GAL+ (266 mg/l). Statistically significant differences were found between the Group DIS+GAL+ and the Group DIS–GAL– (p=0.001), the Group DIS+GAL+ and the Group DIS–GAL–MET– (p=0.02), the Group DIS+GAL+ and the Group DIS–GAL–MET– (p=0.027), the Group DIS+GAL+ and the Group DIS–GAL–MET– (p=0.049) (Table 3).

Lysozyme levels

The highest value of lysozyme mean concentration was found in the Group DIS–GAL– (6 mg/l) and the lowest in the Group DIS+GAL+ (1.1 mg/l). Statistically significant differences were discovered between the Group DIS+GAL+ and the Group DIS–GAL– (p=0.001), the Group DIS+GAL+ and the Group DIS–GAL–MET– (p=0.002) (Table 4).

Levels of IgA antibodies against HSP 60

The highest mean concentration of IgA antibodies against HSP 60 was found in the Group DIS+GAL+
DISCUSSION

Metals from dental alloys are not a physiological component of a human organism. Undesirable side effects may, therefore, appear in sensitive individuals after dental alloys are placed in their oral cavity (Prochazkova et al. 2004). Oral discomfort is often associated with diverse types of allergies and metal intolerance (Wataha 2000) and this fact was confirmed by the results of this study.

Galvanic cells as well as released metal ions may have local and/or general effects on a human organism. This study has shown that the higher the measured galvanic currents values were, the more often the inflammatory affections of the oral mucosa appeared and the higher the risk of this complication was.

Human saliva plays an important defence role in the oral cavity. The oral tissues are under a long-term influence of metal ions, which are released, in low amounts but continuously due to electrochemical corrosion and abrasion from dental alloys (Wataha 2002; Prochazkova et al. 2006). Dental metal restorations significantly increase a metal ion concentrations in the saliva (Kucerova et al. 2002; Prochazkova et al. 2006). The mucosal immune system plays an important role in the defense mechanisms, this study evaluated mucosal defense markers in the saliva.

It is thus necessary to eliminate galvanic cells from the mouth of sensitive individuals. The positive effect of this treatment was confirmed in previous publications of our research group (Prochazkova et al. 2004; Prochazkova et al. 2006), as well as in this study and our results are in concordance with the previously published article (Lindh et al. 2002). After the galvanic cells elimination the patients subjectively experienced an alleviation of their symptoms, as was confirmed by an objective clinical examination and by determining the levels of salivary immune defense markers.

We can conclude that galvanic currents play role in the development of inflammatory affections, lichenoid changes or metallic pigmentation on the oral mucosa and tongue. The higher the galvanic currents values are, the higher the risk of inflammatory and lichenoid lesions is. Lower values of galvanic currents lead frequently to metallic pigmentation.

After the removal of electro active restorations from the oral cavity of the patients with oral discomfort positive changes in the clinical picture were found: within half a year the majority of the patients claimed an improvement of their health, the inflammatory affections on the oral mucosa healed, while lichenoid changes merely diminished. Metallic pigmentation were the only aspect that did not disappear.

The patients with oral discomfort had different levels of immunologic markers in the saliva. Significantly decreased production of IgA1, IgA2, secretory IgA antibodies and lysozyme, analogous to increased

---

**Tab. 5. AntilgA/HSP 60 levels [mg/l].**

<table>
<thead>
<tr>
<th>antilgA/HSP 60 (mg/l)</th>
<th>Data after logarithmic transformation (Basis = 0.005, y = log10(x+basis))</th>
<th>Geometric mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of tested samples</td>
<td>Mean</td>
</tr>
<tr>
<td>DIS+GAL+</td>
<td>41</td>
<td>-0.56</td>
</tr>
<tr>
<td>DIS-GAL-</td>
<td>44</td>
<td>-0.64</td>
</tr>
<tr>
<td>DIS-GAL-</td>
<td>18</td>
<td>-1.43</td>
</tr>
<tr>
<td>DIS-GAL-MET-</td>
<td>15</td>
<td>-0.99</td>
</tr>
</tbody>
</table>

Significant difference (p-value ≤ 0.05) between Group DIS+GAL+ and Group DIS-GAL- and Group DIS-GAL- and Group DIS-GAL-MET-.

**Tab. 6. Comparison of salivary immune markers in patients before and after removal of electro active restorations.**

<table>
<thead>
<tr>
<th>(mg/l)</th>
<th>IgA 1</th>
<th>IgA 2</th>
<th>sigA</th>
<th>lysozyme</th>
<th>antilgA/HSP 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before removal</td>
<td>Mean</td>
<td>1937</td>
<td>374</td>
<td>352</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>2232</td>
<td>251</td>
<td>253</td>
<td>5.6</td>
</tr>
<tr>
<td>After removal</td>
<td>Mean</td>
<td>3236</td>
<td>716</td>
<td>381</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>S.D.</td>
<td>3427</td>
<td>176</td>
<td>276</td>
<td>10.5</td>
</tr>
<tr>
<td>p-value</td>
<td>0.28</td>
<td>0.02</td>
<td>0.78</td>
<td>0.55</td>
<td>0.05</td>
</tr>
</tbody>
</table>

S.D. - Standard deviation

(0.27 mg/l) and the lowest in the Group DIS-GAL- (0.03 mg/l). Statistically significant differences appeared between the Group DIS+GAL+ and the Group DIS-GAL- (p=0.001), the Group DIS-GAL- and the Group DIS-GAL-MET- (p=0.012) (Table 5).

**Results of electro active dental restorations removal from oral cavity**

After the removal of electro active restorations from the oral cavity, 93% of the patients subjectively claimed that their health improved, while morphologic symptoms objectively disappeared in 70 per cent of the treated patients.

Comparing the levels of immunologic markers in the saliva before and after the removal of electro active dental metal restorations from the oral cavity a significant increase in IgA2 levels, rising from the prior concentration of 374 mg/l to the concentration of 716 mg/l (p=0.0206), was found. A non-significant increase in IgA1, sigA and lysozyme levels was also registered.

Comparing the levels of IgA antibodies against HSP 60 in the saliva before and after the removal of electro active dental metal restorations from the oral cavity a significant decrease in IgA against HSP 60 levels (p=0.0496) was found (Table 6).
levels of IgA against HSP 60, played an important role in immune reactions of patients with oral discomfort. After the removal of electro active restorations from the oral cavity of patients with oral discomfort, a significant increase of IgA2 levels in saliva and increase of IgA1, sIgA and lysozyme levels were observed. A significant decrease of IgA against HSP 60 levels was found at the same time.

The results of this study confirmed the importance of galvanic currents in the patients with symptoms of oral discomfort. Measurements of galvanic phenomena in these patients and a subsequent electro active restorations removal should become a common therapeutic procedure, especially in the patients with inflammatory and lichenoid changes on the oral mucosa and tongue.

Any restoration showing a galvanic current over 5 μA can be described as an electro active restoration. A decision to remove such a restoration is governed by the clinical status of a patient. Restorations showing galvanic currents over 10 μA should always be removed in case of patients with oral discomfort.

ACKNOWLEDGEMENTS

The study was supported by the research projects NT 13087-3 and NT 14164-3 of the Internal Grant Agency, Ministry of Health, Czech Republic and by Research Programme PRVOUK-P28/LF1/6 of Ministry of Education, Youth and Sports, Czech Republic.

REFERENCES


