Monitoring of selenium in oral cavity argyria – a clinical and microscopic study

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Abstract

OBJECTIVE: Argyria is generally classified as localized or generalized condition. Distinct pigmentation of the oral mucosa in the vicinity of amalgam fillings is often referred to as amalgam tattoos. Pigmented areas can also be associated with silver-containing corrosion products of dental alloys used for prosthetic restorations. Silver-containing electron dense particles (Ag-EDPs) are frequently found in pigmented areas. We attempted to correlate results of the elemental composition of Ag-EDPs with excerpts from health profiles of our study participants.

DESIGN/SETTING: Eight patients with diagnosed signs of localized argyria were investigated in this study. Biopsies from distinctly pigmented gingival areas were subjected to histological examination, electron microscopy and x-ray microanalysis.

RESULTS: Elemental composition of Ag-EDPs determined by x-ray microanalysis showed mainly silver in combination with sulfur or selenium or a combination of both chalcogens. Elemental analyzes results of Ag-EDPs were analyzed along with excerpts from the patient’s clinical records. Two patients with low or undetectable selenium in the Ag-EDPs suffered from autoimmune thyroiditis, Parkinson’s disease, bronchial asthma, and allergies to molds, pollen and dust.

CONCLUSIONS: We suggest that selenium in Ag-EDPs is a product of the detoxification process for Ag+ ions in gingival tissue and that it may reflect the availability of endogenous selenium for physiological processes in the human body. Its presence or absence might thus be used as another marker of a patient’s health status.

Abbreviations:
EDX - Energy dispersive X-ray microanalysis
TEM - Transmission electron microscopy
STEM - Scanning transmission electron microscopy
Ag-NPs - Silver nanoparticles
EDPs - Electron dense particles
Ag-EDPs - Silver-containing electron dense particles
INTRODUCTION

Argyria, characterized by an irreversible bluish-gray pigmentation of the skin and mucous membranes, is generally classified as localized or generalized (Greene & Su 1987). It is thought that localized argyria is caused by direct external contact with silver. On the other hand, generalized argyria, caused by elevated concentrations of silver in blood, is recognized as a widespread pigmentation of the skin, eyes, and nails (Drake & Hazelwood 2005). Silver sulfide deposits in the skin of patients with argyria are usually detected using x-ray microanalysis and other micro-analytical approaches (Jonas et al. 2007; Sato et al. 1999).

Distinct pigmentation of the oral mucosa in the vicinity of amalgam fillings is often referred to as amalgam tattoos. They are also classified into the category of non-melanin-associated pigmented lesions of the oral cavity (Meleti et al. 2008). Although amalgam is generally considered as the cause of this pigmentation (when small particles are implanted into oral soft tissues during dental procedures (Meleti et al. 2008; Harrison et al. 1977; Lau et al. 2001)), other pigmented areas can be also associated with silver-containing corrosion products of dental alloys used for prosthetic restorations. In this case, they are usually referred to as “metallic pigmentation” (Venclikova et al. 2006; Venclikova et al. 2007). The biocompatibility issues of dental casting alloys, systemic and local toxicity, allergy, and carcinogenicity of released metal ions including silver has been reviewed (Wataha 2000). It is generally thought, that pigmentation caused by silver is harmless from the point of view of inflammation and foreign body reactions (Aoyagi & Katagiri 2004; Martín et al. 2005; Aoyagi & Iwasaki 2008). On the other hand, excepts for mercury, silver has been shown to be the most toxic metal used in dental restoration (Yang & Pon 2003).

Recently, we reported that formation of soluble silver compounds in the gingival sulcus area or in crevices between a crown and the cast post and core reconstruction is possible, and might facilitate the transport of silver compounds into soft tissues (Joska et al. 2009). Silver ion toxicity in cells and organisms is generally acknowledged and it has recently come to the forefront with the boom of silver nanoparticles (Ag-NPs) technologies. Silver ions exert cytotoxic effects on fibroblasts, endothelial cells (Hidalgo & Domínguez 1998), monocytes (Wataha et al. 2002) and other cells and organs, for a review see Panyala et al. (2008). Both Ag+ ions and Ag-NPs can induce apoptosis and necrosis of human monocytes. However, it is still not clear whether Ag+ ions might produce free radicals directly or indirectly through depletion of antioxidant defense mechanisms via interactions with protein thiol groups (Foldbjerg et al. 2009). Quite recently it was shown that Ag+ ions induced oxidative stress-related peroxidase 1 and catalase expression in human hepatoma cells (Kim et al. 2009). Environmental and predisposing genetic factors play a crucial role in the development of systemic autoimmune diseases. The possibility of silver-induced B cell activation and anti-nucleolar autoantibody production has been reported in mice (Abedi-Valugerdi 2009) and the role of certain cytokines and co-stimulatory molecules on the induction of systemic autoimmunity with Ag was assessed in metal-susceptible mice mutants (Havarinasab et al. 2009).

In this study we deal with the elemental composition of particles found in gingival pigmented areas, the types of metallic dental prosthetic reconstruction in the oral cavity of our patients, and their general health profiles. We found that patients with undetectable selenium in their gingival metallic pigmentional areas also suffered from serious diseases, e.g. autoimmune thyroiditis, bronchial asthma and Parkinson’s disease.

MATERIAL AND METHODS

Group of patients

Eight patients were investigated in this study. The group consisted of four men and four women (21 to 71 years old; mean age = 55.1 years). An extensive anamnesis was taken from all patients including their preceding contacts with health care institutions. After being informed of the purpose of this study, they gave their informed consent. Examination of the oral cavity was focused on the location and number of pigmented areas. The composition of dental alloys adjacent to pigmented areas was verified from individual dental records. Panoramic X-rays were taken of all patients.

Transmission electron microscopy (TEM) and Energy dispersive X-ray microanalysis (EDX)

In general, a sample preparation, electron microscopy and energy dispersive X-ray microanalysis were done as described previously (Venclikova et al. 2007). In brief, biopsies of pigmented gingiva were fixed in 2.5% buffered glutaraldehyde for two hours and cut in two pieces. One half was post-fixed in 1% osmium tetroxide. Ultrathin sections mounted on standard copper grids or special plastic grids were analyzed using a Philips CM12/STEM electron microscope equipped with an EDAX DX4 X-ray analysis system (STEM bright-field mode at 80 kV). Ultrathin sections on copper grids contrasted with uranyl acetate and lead citrate (Reynolds 1963) were used as controls.

RESULTS

Six of the patients were diagnosed as having one pigmented area in oral cavity, while the other two had two distinct pigmented areas. In one incidence, the pigmentation was found in the proximity of an amalgam filling and resembled an amalgam tattoo. In the other nine incidences, the pigmentation was localized in the vicinity of a metallic dental prosthetic reconstruction (Figure 1, Table 1). Histological examination of biop-
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Tab. 1. Composition of silver-containing EDPs from gingival pigmented areas and patients anamnesis excerpts.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Clinical symptoms, excerpt from anamnesis</th>
<th>Gingiva pigmented areas characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Patient 1</td>
<td>F</td>
<td>45</td>
<td>Hypertension</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Patient 2</td>
<td>F</td>
<td>21</td>
<td>Without health problems</td>
<td>1</td>
</tr>
<tr>
<td>Patient 3</td>
<td>M</td>
<td>58</td>
<td>Autoimmune thyroiditis, hypacusia</td>
<td>1</td>
</tr>
<tr>
<td>Patient 4</td>
<td>F</td>
<td>69</td>
<td>Allergies (cosmetics, food)</td>
<td>2</td>
</tr>
<tr>
<td>Patient 5</td>
<td>M</td>
<td>57</td>
<td>Hypertension, gout</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Patient 6</td>
<td>M</td>
<td>58</td>
<td>Occupational exposure, allergies (molds, pollen, dust), asthma bronchiale, Parkinson’s disease</td>
<td>1</td>
</tr>
<tr>
<td>Patient 7</td>
<td>M</td>
<td>71</td>
<td>Coxarthrosis, prostate gland hyperthrophy, hypertension</td>
<td>1</td>
</tr>
<tr>
<td>Patient 8</td>
<td>F</td>
<td>62</td>
<td>Ulcus duodeni, allergy (insect sting)</td>
<td>1</td>
</tr>
</tbody>
</table>

AF = amalgam filling, powder in % (wt): Ag 70, Sn 22, Cu 4, Hg 4, mixing ratio powder/Hg = 1:1; AgSn alloy used for post and core reconstruction in % (wt) = Ag 89, Sn 9; AuAgCu alloy used for veneer crown in % (wt) = Au 65, Ag, 20, Cu 0.6, Pd 3; NiCrMo alloy used for metal-bound porcelain crown in % (wt) = Ni 65, Cr 22.5, Mo 9.5; Ag-EDPs = silver-containing electron-dense particles.

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Fig. 1. A typical pigmentation (non-melanin-associated pigmented lesion) in the vicinity of metallic dental prosthetic reconstruction.

DISCUSSION

Silver-containing dental alloys used for teeth restorations may release silver under conditions typically found in the oral cavity (Schmalz et al. 1998; Schmalz & Garhammer 2002); for review see Geurtsen (2002); we demonstrated this recently in the gingival sulcus area.
and in crevices between a crown and the cast post and core reconstruction (Joska et al. 2009). In this study, we report on silver containing particles containing sulfur or selenium or both chalcogens, in pigmented lesions of the oral cavity. We failed to observe massive tissue impregnation with EDPs such as those reported by Zhang et al. (2007) and never found amalgam related mercury (Venclikova et al. 2006). In our previous study (Joska 2009) we described occurrence of Ag-EDPs ranging in size from 10 to 310 nm in metallic pigmentation of the gingiva. This finding suggests that small particles are forced to aggregate into larger ones as their surface-to-volume ratio decreases with particle size. This results in a smaller interactive surface for foreign bodies localized in cells and tissues. EDPs found in the pigmented gingival lesions of our patients, contained mostly silver, which corresponds well with the elemental composition of dental alloys used for prosthetic dental reconstruction. Selenium was found only in Ag-EDPs and never in Ag-free EDPs or in the surrounding tissues. Similar observations have also been reported by other authors (Aoyagi & Katagiri 2004; Garhammer et al. 2003).

Some authors reported finding only silver-sulfur containing EDPs in amalgam tattoos or in metal pigmentation (Aoyagi & Katagiri 2004; Aoyagi & Iwasaki 2008), while others also found selenium in them (Rechmann 1994; Zhang et al. 2007). Silver and sulfur in granules and with other elements (Se, Hg, Ti and Fe) were detected using X-ray microanalyses of skin biopsies from five patients with occupational argyria (Bleehen et al. 1981). However, the presence of Se, Hg, Ti and Fe was explained as a probable result of occupational exposure. Sulfur as a consistent part of “silver-laden granules” in argyria originating from remnants of acupuncture with silver needles has been described in two cases (Sato et al. 1999), but selenium was confirmed in only one case. Silver-sulfur-selenium deposits in the connective tissue of the submucosa were observed in one patient with nasal argyria, but again the Se occurrence was ascribed to occupational exposure (Se was a component of the toning agent used in photographic development process at patient’s workplace (Naqvi et al. 2007)). Nevertheless, the presence of selenium in silver containing inclusions has already been discussed as a product of the detoxification processes (Matsumura et al. 1992; Rechmann 1994; Sato et al. 1999) that play a role in development of argyria and amalgam tattoos, respectively. General mechanisms of transition metal (Ag, Cd and Hg) detoxification in the bloodstream, in which selenium and selenoprotein P are involved, has already been suggested by Sasakura & Suzuki (1998). The detoxification model of heavy metals (Hg, Ag, Cu, and Cd) taking place in the liver of marine mammals and seabirds that involves selenium, has also been proposed (Ikemoto et al. 2004). The sequestration of Ag⁺ by selenium represents an efficient natural detoxification mechanism resulting, however, in the physiological inactivation of selenium. It might thus be unavailable for other physiological processes. The role of selenium in anticancer prevention was recently reviewed by Schrauzer (2009).

Some authors, in addition to the toxic effects of silver, point out its involvement in immunopathological processes (Bigazzi 2000). Frequency of hypersensitivity to silver among 700 symptomatic, metal-exposed
patients was estimated to about 2.7% (Valentine-Thon et al. 2006). Signs and symptoms of allergies to metals caused by amalgam tattoos might be systemic and not just local (Pigatto et al. 2006; Panyala et al. 2008). Therefore, the influence of metal ions on the human organism might vary greatly and not everyone might tolerate or reacts to it in the same way (Geurtsen 2002).

Two our patients had practically undetectable content of selenium in their Ag-EDPs originating from pigmented areas. Interestingly, both patients suffered from severe diseases with complications including autoimmune thyroiditis in one case (Table 1, patient 3) and bronchial asthma and Parkinson’s disease in the other one (Table 1, patient 6). We suggest that selenium in Ag-EDPs is a product of the detoxification process for Ag+ ions in gingival tissue and that it reflects the availability of endogenous selenium and/or selenium containing organic compounds for physiological processes in the human body. Its presence or absence may thus be an indicator of patient’s health status. However, the correlation between selenium content in Ag-EDPs and the clinical manifestations of the above mentioned diseases will require further detailed studies.

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Selenium and localized argyria in the oral cavity


