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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 paticipants.
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 pigmentations 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 (Venclíkova *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-

Tab. 1. Composition of silver-containing EDPs from gingival pigmented areas and patients anamnesis excerpts.

Patient	Sex	Age (years)	Clinical symptoms, excerpt from anamnesis	Gingiva pigmented areas characteristic		
				No.	Materials used for adjacent reconstruction	Ag-EDPs composition
Patient 1	F	45	Hypertension	1	AF	Ag, S, Se
				1	AgSn, AuAgCu	Ag, S, Se
Patient 2	F	21	Without health problems	1	AF, NiCrMo	Ag, S, Se
Patient 3	M	58	Autoimmune thyreoiditis, hypacusia	1	AgSn, acrylic crown	Ag, S>>>Se
Patient 4	F	69	Allergies (cosmetics, food)	2	AgSn, acrylic crown	Ag, S, Se
Patient 5	M	57	Hypertension, gout	1	AgSn, acrylic crown	Ag, S, Se
Patient 6	M	58	Occupational exposure, allergies (molds, pollen, dust), astma bronchiale, Parkinson's disease	1	AgSn, AuAgCu	Ag, S>>>Se
Patient 7	M	71	Coxarthrosis, prostate gland hyperthrophy, hypertension	1	AgSn, AuAgCu	Ag, S, Se
Patient 8	F	62	Ulcus duodeni, allergy (insect sting)	1	AgSn, AuAgCu	Ag, S, Se

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 a**lloy used for veneer crown in % (wt) = Au 65, Ag, 20, Cu 9.6, 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.

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

sies from pigmented areas showed stratified squamous epithelium with an underlying lamina propria gingivae. Electron microscopy of ultrathin sections revealed numerous submicroscopic electron-dense particles (EDPs) of various sizes and location within the lamina propria. EDPs occurred alone and in clumps, extracellularly and intracellularly, for details see Venclikova *et al.* (2006). EDPs were never observed in the epithelium.

Elemental composition of EDPs was determined by x-ray microanalysis (Figure 2). Silver-containing EDPs (Ag-EDPs) of ovoid shape were identified in ultrathin sections from all examined biopsies (Table 1). The presence of silver was accompanied by sulfur or selenium. However, a considerable amount of the analyzed Ag-EDPs contained both chalcogens (Table 1, Figure 2A). Although the Ag-S-Se, Ag-S and Ag-Se EDPs were typical for pigmented areas, other kinds of electron-dense particles containing at least one of the following elements Cu, Sn, Ti, Zn, Fe, Al, Ti, Cr, Mg, Si, Ca and P were occasionally discovered (Venclikova *et al.* 2007). In no case, however, was mercury detected.

The results of elemental analysis, of the Ag-EDPs found in the pigmented areas in the oral cavity, were analyzed with respect to excerpts from the patient's clinical records (Table 1). Surprisingly, the two patients with undetectable selenium content in the Ag-EDPs (Patient 3 and Patient 6, Table 1, Figure 2B) suffered from serious diseases such as autoimmune thyroiditis, Parkinson's disease, bronchial asthma, and allergies to molds, pollen and dust.

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).

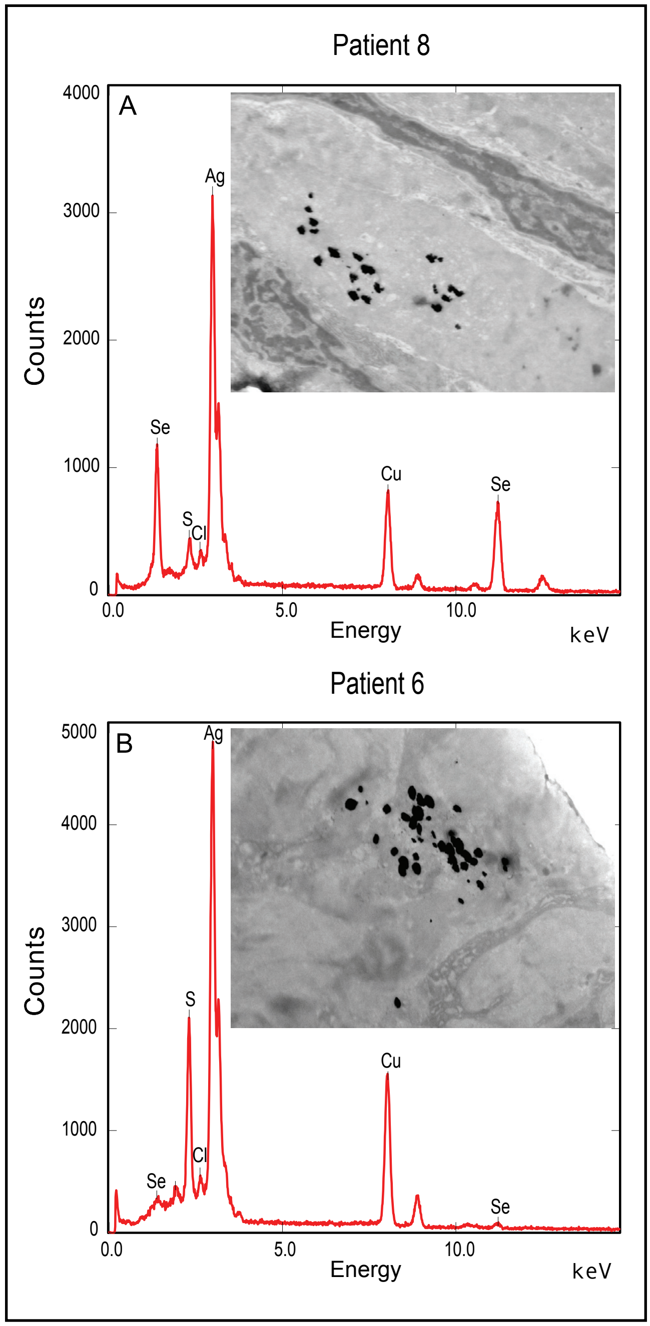

Fig. 2. Characteristic spectra obtained from silver-containing electron-dense particles (Ag-EDPs) found in biopsies from pigmented areas (the inserts). Ultra-thin sections were mounted onto copper grid (artificial Cu peaks in spectra); A - typical spectrum from Ag-EDPs – patient 8, Table 1; B - spectrum from Ag-EDPs with very low content of selenium – patient 6, Table 1.

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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