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SF-ICP-MS Analysis of Palladium in Fluids of Patients Sensitized to Metal-Based Dental Restorations

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Abstract: Palladium (Pd) belongs to the platinum group elements and its use has more than doubled in the past ten years. Encouraged by positive patch test results in some patients suffering from oral syndromes, Pd was included in the routine patch testing for contact dermatitis. In the present study, differences in the trend of sensitization to Pd in a contact dermatitis clinic population between 1996 and 2006 were evaluated. Since many *in vitro* studies have confirmed the corrosion of dental alloys and indicated that metal components are released in the oral cavity, Pd content was examined in leaching solutions from dental prostheses and in samples of saliva, serum and urine of subjects with adverse reaction to dental prostheses. Measurements were performed by means of Sector Field Inductively Coupled Plasma-Mass Spectrometry (SF-ICP-MS). Results showed that the frequency of mono-reactions to Pd are increased from 1996 (0.11%) to 2006 (1.6%), suggesting that this metal is becoming a "new" potential allergen. The mean values of Pd levels detected in urine were higher than those found in saliva and in serum. All biological samples collected from patients showed significantly higher levels of Pd than controls (saliva, 255 ng/L *vs* 11; serum, 60 *vs* 15; urine, 580 *vs* 20). Moreover, after removal of the prostheses a consistent reduction of Pd content in fluids was observed.

Keywords: Palladium, dental restoration, dental alloys, contact dermatitis, SF-ICP-MS, saliva, urine, serum.

1. INTRODUCTION

Palladium (Pd) is a metal of the platinum group, the output and use of which has more than doubled in the past ten years [1, 2]. Pd has emerged as a suitable substitute, lowering the costs of raw materials and manufacture, in industry, in alloys for relays and switching systems in telecommunications equipment, as a catalyst in white gold, for electrical switches in aircraft, and in most cast dental restorations and amalgams (dental alloys may contain up to 10% Pd) [3-7]. Moreover, data on local [8] and systemic toxicity [9] seemed to prove Pd safety. Due to its relatively cheap price, suitable processing qualities, and due to the fact that dentistry alloys containing Pd appear more resistant to mechanical wearing effects [10, 11], exposure to Pd from use of dental appliances become more frequent in the population.

This increasing demand is also attributed to the emanation of the European Nickel Directive 94/27/EC [12-14] restricting the use of nickel (Ni) in all industrial products placed in direct and prolonged contact with the skin for limiting the consumer's contact with this potential allergen. This shift away from the use of Ni has led to an increase in the use of different metal compounds for specific destinations such as the alloys used in orthopaedics, in Ni-free jewellery [4] and in dental appliances [15].

Up to date, it has been felt that Pd is a low-risk metal for dentistry because of its low rate of dissolution; nevertheless, despite the fact that dermatitis caused by Pd was previously considered rare [5], dermatitis caused by Pd has recently increased [1, 3, 16, 17] with a significant number of patients allergic to Ni sulphate who have also shown positive patch-test reactions to Pd chloride [18, 19], either in cases associated with allergic contact dermatitis (ACD), oral symptoms [20, 21], or non-mucosal dermatitis [22]. In the past decade, several reports have been published on sensitivity to Pd describing general symptoms like contact allergy of the oral mucosa [21] such as cheilitis, stomatitis [23], burning mouth, lichenoid reactions, orofacial granulomatosis, or welling of the lips and cheeks, dizziness, asthma, chronic urticaria [1, 24, 25]. In some case reports, complaints disappeared after replacement with Pd-free constructions [26].

Sensitization to Pd chloride reached the highest percentage (9.7%) in Italy during the year 2000 [1]. Recently other different studies reported a sensitization rate of 9.4% in England [27], 8% in Europe [28], and 8.3% in unselected eczema patients in Austria [16], where Pd has started to displace amalgam in dental fillings because of concerns about mercury toxicity, and gold due to price factors.

In light of this background it can be assessed that these sensitizations might be the first indicator of an emerging problem. Therefore, encouraged by positive patch test results in some patients suffering from burning mouth syndrome, in 1996 Pd was included in the routine patch testing for contact dermatitis performed at the Department of Allergology of the San Gallicano Dermatology Institute. The present study was undertaken with the aim to analyse the trend of sensitization to Pd in a contact dermatitis clinic population during a 10-year period (1996-2006). Furthermore, since many *in vitro* studies have confirmed the corrosion of dental alloys and indicated that metal components are released in the oral cavity

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[29, 30], the Pd content was examined in saliva, serum and urine samples collected from selected subjects with suspected adverse reaction to dental prostheses. Moreover, a leaching test and a micro morphological imaging were performed on orthodontic prostheses. The determination of Pd in biological samples requires analytical techniques of adequate detection power. This poses substantial problems to the majority of instrumental analytical techniques, such as Electrothermal Atomization Atomic Absorption Spectrometry (ETA-AAS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Therefore, an analytical method for the determination of Pd in saliva, urine and serum was developed. Measurements were performed by means of Sector Field Inductively Coupled Plasma-Mass Spectrometry (SF-ICP-MS), well established as a powerful analytical technique for the determination of trace and ultratrace elements in environmental and biological samples. Since polyatomic and isobaric interference could constitute a problem in the analysis of Pd in such matrices, an interference study was performed to quantify the phenomenon extent and to apply eventual corrections.

2. SUBJECTS AND METHODS

2.1. Patients

All the subjects consecutively patch tested at the Department of Allergology of the San Gallicano Dermatology Institute during one year in 1996 (n = 3071) and in 2006 (n = 3093) were included in the present analysis. A detailed medical history of patients, including history of metal exposure and wearing of metal dental prostheses, was recorded. Six patients among those observed during 2006, showing a positive patch test reaction to Pd and oral symptoms, including gingivitis, taste irritation, dry mouth, and burning mouth, associated to the presence of metal restorations, except amalgams, were selected for the analysis of Pd content in biological specimens. Samples from 10 subjects with negative patch test and without any kind of metal restorations or amalgams were also collected for background metal content determination. In one patient, the levels of Pd were compared in the biological fluids collected before and one month after the removal of the dental prostheses. All subjects were duly informed about the purpose of the study and their consent was obtained.

2.2. Biological Samples

Salivary fluid, urine and serum samples were collected from each patient and control subject. Saliva (1 mL) was collected in polyethylene (PE) tubes (Greiner, Frickenhausen, Germany) before breakfast, tooth brushing, and smoking using. This procedure was repeated after 2 days. The samples were treated with 1 mL of ultra pure HNO_3 (Carlo Erba, Milan, Italy) in a microwave oven at 80 °C for 1 hr (FKV Milestone, Bergamo, Italy), diluted up to 10 mL with deionised water (EasyPure, PBI International, Milan, Italy) and subsequently analysed. Serum and urine samples were collected in the morning in PE tubes and simply diluted (1:5 v/v) with deionised water.

2.3. Patch Testing

Skin patch tests were performed by applying Finn Chambers with haptens on unaffected skin of the upper backs of the subjects using the European Standard Series of contact allergens (Hermal Trolab, Reinbeck, Germany) supplemented with 1% palladium chloride ($PdCl_2$) applied in petrolatum. Patch test responses were examined after two days and defined according to the International Contact Dermatitis Research Group guidelines [31].

2.4. SF-ICP-MS Analysis

Measurements of Pd content in all biological samples were performed by SF-ICP-MS. Characteristics and settings of the analytical instrumentation are resumed in Table **1**. The selection of the analytical isotopes ^{105}Pd, ^{106}Pd and ^{108}Pd out of those available was dictated by their higher abundance. The potential interferences on these masses are reported in Table **2**.

Table 1. Instrumental Settings and Data Acquisition Parameters

Apparatus	Element (Thermo-Finnigan, Bremen, Germany) equipped with the Guard Electrode device
RF power	1250 W
Sample introduction	Nebulizer: concentric, Meinhard glass type; Spray chamber: Scott-type, water-cooled
Internal standard	^{115}In
Analytical Masses	^{105}Pd, ^{106}Pd, ^{108}Pd
Interface	Pt cones
Gas flow rates (l/min)	Cooling, 14; auxiliary, 1.00; nebulizer, 1.10
Resolution (m/Δm)	Medium resolution = 3000
Acquisition mode	Electric scan
Number of scans	15

Table 2. Potential Interferences on Pd Analytical Isotopes

Analytical Mass and Abundance (%)	Potential Interference
^{105}Pd (22.3)	$^{209}Bi^{++}$, $^{65}Cu^{40}Ar$, $^{67}Zn^{38}Ar$, $^{69}Ga^{36}Ar$, $^{89}Y^{16}O$, $^{88}Sr^{17}O$, $^{87}Sr^{18}O$, $^{85}Rb^{18}O$, $^{104}Pd^{1}H$, $^{104}Ru^{1}H$, $^{93}Nb^{12}C$, $^{92}Zr^{13}C$, $^{92}Zr^{13}C$, $^{90}Zr^{15}N$, $^{91}Zr^{14}N$, $^{89}Y^{15}N^{1}H$, $^{90}Zr^{14}N^{1}H$, $^{88}Sr^{16}O^{1}H$, $^{87}Rb^{17}O^{1}H$, $^{86}Kr^{18}O^{1}H$, $^{86}Sr^{18}O^{1}H$, $^{86}Sr^{19}F$, $^{74}Ge^{31}P$, $^{74}Se^{31}P$, $^{72}Ge^{33}S$, $^{73}Ge^{32}S$, $^{68}Zn^{37}Cl$, $^{70}ZN^{35}Cl$
^{106}Pd (27.3)	^{106}Cd (1.25), $^{66}Zn^{40}Ar$, $^{68}Zn^{38}Ar$, $^{70}Ga^{36}Ar$, $^{70}Zn^{36}Ar$, $^{89}Y^{17}O$, $^{88}Sr^{17}O$, $^{88}Sr^{18}O$, $^{105}Pd^{1}H$, $^{93}Nb^{13}C$, $^{94}Zr^{12}C$, $^{94}Mo^{12}C$, $^{92}Zr^{14}N$, $^{92}Mo^{14}N$, $^{89}Y^{16}O^{1}H$, $^{88}Sr^{18}O^{1}H$, $^{87}Sr^{17}O^{1}H$, $^{87}Rb^{18}O^{1}H$, $^{87}Sr^{19}F$, $^{87}Rb^{19}F$, $^{75}As^{31}P$, $^{72}Ge^{34}S$, $^{74}Ge^{32}S$, $^{73}Ge^{33}S$, $^{74}Se^{34}S$, $^{69}Ga^{37}Cl$, $^{71}Ga^{35}Cl$
^{108}Pd (22.6)	^{108}Cd (0.89), $^{68}Zn^{40}Ar$, $^{70}Ge^{38}Ar$, $^{72}Ge^{36}Ar$, $^{92}Zr^{16}O$, $^{92}Mo^{16}O$, ^{90}Zr ^{18}O, $^{107}Ag^{1}H$, $^{96}Mo^{12}C$, $^{96}Ru^{12}C$, $^{96}Zr^{12}C$, $^{95}Zr^{13}C$, $^{93}Nb^{15}N$, $^{94}Mo^{14}N$, $^{94}Zr^{14}N$, $^{93}Nb^{14}N^{1}H$, $^{91}Zr^{16}O^{1}H$, $^{90}Zr^{17}O^{1}H$, ^{89}Y $^{18}O^{1}H$, $^{89}Y^{19}F$, $^{77}Se^{31}P$, $^{74}Ge^{34}S$, $^{74}Se^{34}S$, $^{74}Se^{34}$ S, $^{74}Se^{34}S$, $^{74}Se^{34}S$, $^{76}Se^{32}S$, $^{75}As^{33}S$, $^{76}Ge^{32}S$, $^{71}Ga^{37}Cl$, $^{73}Ge^{35}Cl$

The analytical mass spectra were investigated at medium resolution (m/Δm 3000) to study in detail the behaviour of these molecular ions. Increasing concentrations of the interfering elements were added to each matrix to match and even exceed their expected ranges of concentration. Among all possible interferences, some of these were particularly heavy because of the high content of the relevant element (Cl, Cu, F, Rb, Sr and Zn) in the biological samples and the influence on the analytical mass signals could not be disregarded. In fact, for ^{105}Pd particular attention was given to the following species: ^{65}Cu^{40}Ar, ^{88}Sr^{16}O^{1}H, ^{86}Sr^{19}F, ^{68}Zn^{37}Cl and ^{70}Zn^{35}Cl. Whilst, the interferences from ^{67}Zn^{38}Ar, ^{88}Sr^{17}O, ^{87}Sr^{18}O, ^{85}Rb^{18}O and ^{87}Rb^{17}O^{1}H were improbable, due to low isotope abundance of ^{38}Ar (0.06%), ^{17}O (0.04%) and ^{18}O (0.2%). As concern the ^{106}Pd, the molecular ions that could influence the analytical signal were: ^{106}Cd (abundance, 1.25%), ^{66}Zn^{40}Ar, ^{87}Sr^{19}F and ^{87}Rb^{19}F, whilst the contributes of ^{88}Sr^{17}O, ^{88}Sr^{18}O, ^{88}Sr^{18}O^{1}H, ^{87}Sr^{17}O^{1}H, ^{87}Rb^{18}O^{1}H were of moderate intensity. Finally, the ^{108}Pd was hampered principally by ^{68}Zn^{40}Ar and ^{108}Cd (abundance, 0.89%). The remaining interfering species, listed in Table **2**, were not important because of the very low concentration of As, Ga, Ge, Kr, Mo, Nb, Ru, Se, Y and Zr.

The calibration of the technique was performed by the adoption of the standard addition mode: diluted single-element standards were added to the analytical solutions of saliva, serum and urine. To compensate the instrumental drifts and matrix effects, indium was added as the internal standard (IS) to each sample.

The limits of detection (LoDs) were calculated on the basis of the 3-σ criterion on saliva, urine and serum obtained from non-exposed subjects. The same samples, spiked as needed, were used for the intra-day imprecision tests. Because of the lack of reference materials with certified levels of Pd for the matrices under study, recovery tests were carried out to assess the accuracy of the method. To this purpose, a solution containing Pd was spiked to biological samples, with aliquots of 10, 50 and 100 ng/L of the analyte prior to pre-analytical treatment and then analyzed.

2.5. Leaching Experiments

A leaching test on orthodontic prostheses was performed in order to evaluate the release of Pd in saliva. Two different metallic pivots and bridge-work crowns were leaved in contact with samples of saliva - taken from two subjects of the control group not-exposed to Pd-containing prostheses - in a thermostatic bath at 37 °C for 24 hours. After incubation, saliva samples were digested and analyzed as mentioned above. Samples were tested in duplicate.

2.6. 3D Imaging of Dental Prostheses

In order to examine the degradation of dental prostheses, the analysis of the external and internal micro morphology of orthodontic prostheses was performed using computerized X-ray micro tomography. The hardware device used in this study was a desktop X-ray micro focus CT scanner (SkyScan 1072, SkyScan bvba, Aartselaar, Belgium).

3. RESULTS

3.1. Patch Testing

In 1996, out of the 3071 patients tested with clinical evidence of eczema, 897 (29.2%) showed a positive reaction to at least one allergen. The subjects had the following characteristics: 653 women; 244 men; age range between 21 and 75 years (average, 43). In 2006, among the 3093 patients, 928 (30%) developed at least a positive reaction to patch test. The subjects had the following characteristics: 676 women; 252 men; age range between 21 and 75 years (average, 39).

Accordingly to our observations, sensitization to Pd chloride alone increased by the years. In fact, a monosensitization to Pd was found in only one patient (0.1%) out of 897 subjects in 1996, and a monosensitization in 15 (1.6%) out of 928 patients in 2006. Characteristics of these patients are resumed in Table **3**. The number of patients with positive patch test responses to Ni alone or polysensitized to Pd and Ni together remained the same between 1996, with 323 cases (36%) positive to Ni alone and 110 (12.3%) to Ni and Pd, and 2006, with 328 subjects (35.3%) positive to Ni alone and 128 (13.8%) to Ni and Pd. In 1996, 463 patients (51.6%) showed a positive reactions to other allergens and the same number in 2006, i.e., 433 patients (48.8%).

3.2. Clinical Observations

Clinical evaluation of Pd-sensitive patients (Table **3**) gave the present results: oral symptoms as cheilitis, perioral dermatitis and periodontitis were observed in the Pd-monosensitized patient in 1996 and in 5 of the 15 Pd-positive patients in 2006; burning mouth syndrome characterized by symptoms of pain or burning sensation of the tongue in absence of any clinical evidence of eczema and/or blistering at the moment of the evaluation was observed in 5 patients; glossodynia was reported from 5 patients sometimes associated with the burning mouth syndrome. Furthermore, mucositis and stomatitis were observed in 4 of these patients. Five patients suffered also from hand dermatitis and diffuse eczema. Anamnestic data showed that all patients monosensitized to Pd were wearing dental prostheses and 4 of them, suffering also from hand dermatitis, were dental personnel (dentists and their assistants).

3.3. SF-ICP-MS Method Validation

The interference study was carried out in order to chose the best isotope mass of Pd and to avoid or overcome the influence of mass interferences. Among the detected Pd isotopes, the mass 108 was chosen as the best because its signal is hampered only by ^{68}Zn^{40}Ar and ^{108}Cd (Fig. **1**). Operating at medium resolution, the interference of the Zn polyatomic species was physically shifted, whereas for the isobaric interference of ^{108}Cd on ^{108}Pd the use of a mathematical correction was necessary and, therefore, the following equation was resorted to:

$$I^{108}\text{Pd} = I108 - [I^{111}\text{Cd x IR}] \quad (1)$$

where I is the intensity (counts) and IR is the isotopic abundance ratio between the masses ^{108}Cd and ^{111}Cd, chosen to quantify the Cd intensity.

Data on LoDs, precision and recovery are listed in Tables **4** and **5**. The detection power achieved by SF-ICP-MS was adequate to analyze the expected concentrations of Pd in the studied matrices. The reliability of the overall treatment and instrumental procedures was proven by the satisfactory precision obtained on ten measurements. At a concentration of 50 ng/L, precision varied from 5.2% for the digested solutions (saliva) to 7.9% for water-diluted solutions (serum and urine). In the latter case, the presence of organic matter may

Table 3. Characteristics and Clinical Symptoms of the 16 Subjects Monosensitized to Pd

Yrs	Patients	Sex	Gingivitis/Periodontitis	Burning Mouth Syndrome	Glossodynia	Cheilitis	Mucositis/Stomatitis	Other*
1996	1	F	×			×		
2006	2	M		×				×
2006	3	M			×		×	
2006	4	F	×					
2006	5	F		×				×
2006	6	F			×			
2006	7	F	×			×		
2006	8	F			×		×	
2006	9	M		×				×
2006	10	F	×			×		
2006	11	M	×				×	
2006	12	M		×				×
2006	13	M			×			
2006	14	F	×					
2006	15	F		×			×	×
2006	16	M			×	×		

* Hand dermatitis and diffuse eczema.

account for a higher (although acceptable) instability in the analytical signal, which cannot be fully counterbalanced by the use of the IS. The results of the recoveries were in good agreement with the added amounts for all matrices.

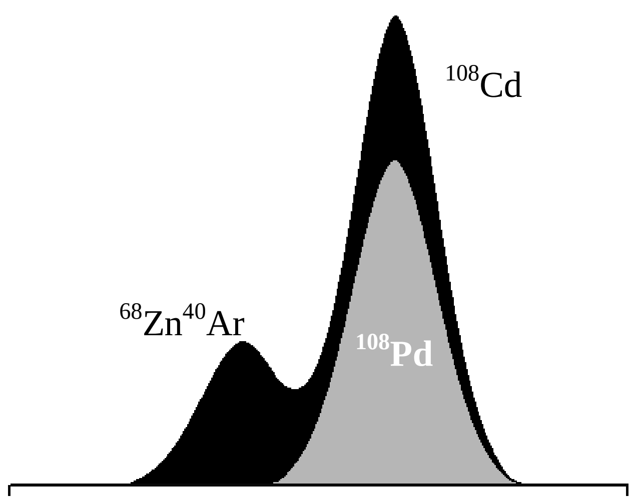


Fig. (1). Interferences profile on mass ^{108}Pd at medium resolution mode ($m/\Delta m$=3000).

Table 4. LoDs (ng/L) and Intra-Day Precision (%) at 50 ng/L Solution for the Quantification of Pd in Saliva, Serum and Urine by SF-ICP-MS

Element	Saliva		Serum		Urine	
	LoD	Precision	LoD	Precision	LoD	Precision
^{108}Pd	5	5.2	12	6.8	8	7.9

3.4. Concentration of Pd in Biological Samples

Palladium contents of saliva, serum and urine samples from patients wearing dental appliances and from the control group are shown in Table **6**. In all biological samples were detected levels of Pd greater than the detection limit. The mean values of Pd levels detected in urine samples were higher than those found in saliva or in serum. All biological samples collected from patients showed significantly higher levels of Pd than those collected from control subjects.

One patient, who had the dental prostheses removed due to severe oral symptoms, showed a consistent reduction of Pd content in saliva (250 ng/L *vs* 150 ng/L), serum (120 ng/L *vs* 50 ng/L) and urine (570 ng/L *vs* 280 ng/L) measured one month after the removal.

3.5. Concentration of Pd in Leaching Samples

During the leaching experiments no colouration of saliva was noticed in any of the samples. Measurable levels of Pd were released in the healthy saliva by all the dental appliances with the following differences: the mean values of Pd released from the two metallic implanted pivots (0.73 ± 0.06 and 0.79 ± 0.05 ng/mL) were higher than those released by the two bridge-work crowns (0.12 ± 0.03 and 0.19 ± 0.02 ng/mL).

3.6. Microcomputed Tomography

The 3D imaging provided precise evidences of structural defects in the microarchitecture of the dental prostheses obtained from the patient suffering of oral symptoms who decided to restore the dental appliance after participating to this study (Fig. **2**). This analysis confirmed that the dental appliance could be susceptible to corrosion.

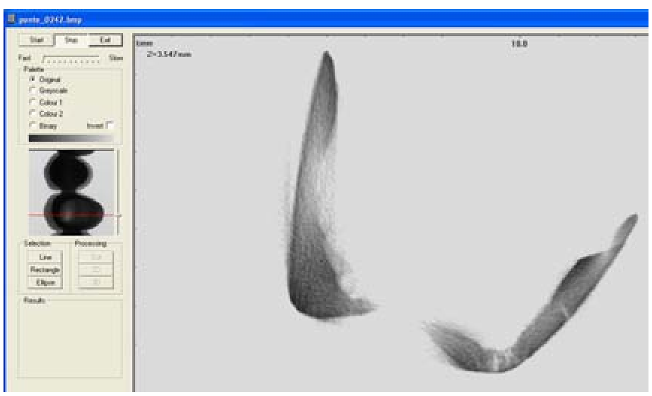
Table 5. Recovery Data for the Determination of Pd in Saliva, Serum and Urine

	Saliva		Serum		Urine	
	Spike (ng/L)	Recovery (%)	Spike (ng/L)	Recovery (%)	Spike (ng/L)	Recovery (%)
Element	10	101	10	98	10	96
^{108}Pd	50	95	50	102	50	98
	100	105	100	103	100	105

Table 6. Pd Content in Saliva Fluid, Urine and Serum by SF-ICP-MS

Specimen	Study Group (n=6)		Control Group (n=10)	
	Pd Concentration (ng/L)		Pd Concentration (ng/L)	
	Mean	Median	Range	Mean
Saliva	255	160	19 - 1140	11
Serum	60	70	8 - 180	15
Urine	580	280	250 - 580	20

(**A**)



(**B**)

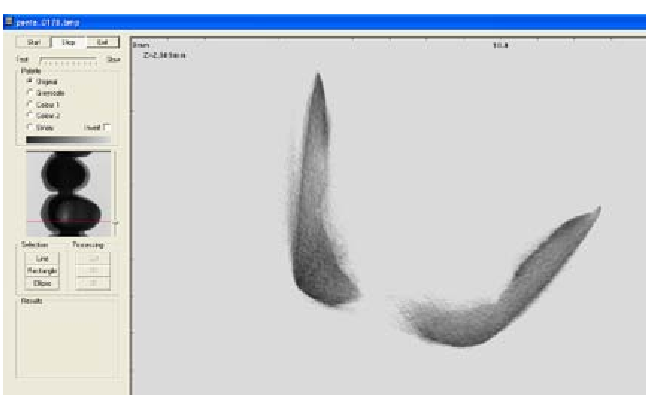


Fig. (2). The Micro-CT 3D images provided evidences of corrosion and structural defects in the micro architecture of the prostheses obtained from the patient. (**A**) dental appliance obtained from the patient. (**B**) a new dental appliance.

4. DISCUSSION

Hypersensitivity to metals is a frequently encountered problem and it could be the cause of significant morbidity to patients. Palladium is a precious metal used in jewellery, industry and in most cast dental restorations [4-7]. Even if this metal was considered safe, since 1990 multiple reports have documented patients with positive patch test reactions to Pd chloride [32]. Sensitization in humans seems to be induced by chronic low dose exposure as documented by Stejskal *et al.* [33] which found specific lymphocytes in patients suffering from local and systemic symptoms attributed to dental restorations. People with burning mouth syndrome were frequently not tested for contact allergy and in earlier tests potentially relevant allergens such as Pd were not included, thus the cause of sensitization might have remained unrecognized [21].

Despite it is well assumed that hypersensitivity to Pd is generally related to a simultaneous sensitization to other metals such as Co, Cr and Ni [1, 5, 19, 34, 35], the present study showed that the frequency of mono-reactions to Pd are increased from 1996 (0.11%) to 2006 (1.6%), suggesting that this metal is becoming a "new" potential allergen since Pd has been used more frequently as a substitute for Ni in the last ten years.

Interestingly, all patients monosensitized to Pd with intra-oral symptoms were wearing dental prostheses at the time of the clinical evaluation. This consideration is in accordance with clinical case reports in which several restorative materials have been reported as a cause of allergic contact mucositis [36, 37] or of the appearance of lichenoid lesions on buccal mucosa that is in direct contact with the dental restoration [38]. In dentistry Pd is a very common component of dental appliances of all types and the buccal mucosa, lateral tongue and gingival are most commonly affected by contact allergic reactions [39]. Even if contact allergy involving the oral mucosa is a poorly understood clinical entity, this and other articles suggest that it may be more common than previously believed [37].

The concomitant presence of oral symptoms, hypersensitivity to Pd alone and the use of dental appliances at the moment of the clinical examination suggested to the authors to analyse if it could be possible to find measurable quantity of Pd in biological fluids from a group of selected patients by means of SF-ICP-MS. This instrumental device is a powerful technique for multielemental analysis of several elements in biological matrices because of its capability of reaching very low-detection limits. The problems of mass interferences arising in ICP-MS are mostly solved by SF-ICP-MS because this technique offers the possibility to operate at increasing steps of resolution. In respect to other analytical techniques, such as ETA-AAS or ICP-AES, this method reached quantification limits lower than one order of magnitude, also operating at the highest resolution where a lowering of the sensitivity is consequential. Finally, a series of proper interference studies and validation procedures permitted to provide the necessary accuracy data. For this reason, the Pd results detected in this study, although at levels much lower than those before achieved with other analytical techniques [40], could be considered reliable and accurate.

In samples of saliva measurable levels of Pd were detected and the amount of Pd in the study group was greater than in the controls. These results support data from Garhammer *et al*. showing that components of dental alloys could be released into saliva [40] apart from accumulate in the adjacent soft tissue of the oral cavity [41].

Although Pd content of saliva showed little variations between subjects of the present study, we took into consideration that individual saliva production, regional pH value in the surrounding area of the prostheses, diet, presence of proteins from food binding the released metals and personal habits (chewing gum use) may influence the corrosion and metal ions release from the dental appliances [29]. In the light of these considerations, our choice to exclude subjects with amalgams was made to avoid metal contamination from materials different from prostheses [40] and the recommendation to avoid eating, tooth brushing or smoking [42] before saliva collecting was made to limit the individual variables between subjects and standardizing saliva collection as consistently as possible.

The serum concentrations of Pd, found in subjects of the study group, were rather low and did not reflect Pd levels found in salivary fluids and urinary excretions, confirming the results obtained in a metabolism study of Pd in rats following sub-acute oral exposure [43]. In that study, serum Pd concentration did not correlate with the dose of Pd administered to rats and the levels were lowest also in that case.

The mean values of Pd in urine samples were the highest. Also this trend is in agreement with the results seen in the same work cited above. These data are in accordance with the fact that preferentially, the pathway of Pd in the organism shows a predominance of excretion (through urine and faeces) connected to an accumulation in the kidney [44].

Even if the Au–Pt–Pd dental alloys are considered the most resistant materials to electrochemical and chemical corrosion, numerous parameters are not always controllable such as the quality of the restoration and the habits of life of the patients. The amount of Pd that is biologically available in the form of Pd ions depends on the corrosion behaviour of Pd-containing dental alloys in the mouth.

In accordance with other *in vitro* studies [40, 45, 46], we demonstrated, by performing the leaching experiments, that elemental release of metal ions from various types of dental casting alloys could be possible and we also confirmed the presence of defects in the dental appliances looking at the images obtained by the microcomputed tomography.

CONCLUSIONS

In conclusion, the slight increase of Pd monosensitization observed in our patients could be due to the large use of Pd in dental appliances rather than to the environmental exposure. In fact, patients monosensitized to Pd showed clinical symptoms associated with measurable levels of Pd in all biological fluids tested. Interestingly, the patient who had the dental prostheses removed due to severe oral symptoms presented a consistent reduction of Pd content in biological fluids associated to a clear clinical improvement.

ABBREVIATIONS

ACD	=	Allergic contact dermatitis
CT	=	Computer tomography
ETA-AAS	=	Electrothermal atomization atomic absorption spectrometry
ICP-AES	=	Inductively coupled plasma atomic emission spectometry
ICP-MS	=	Inductively coupled plasma mass spectrometry
IR	=	Isotopic abundance ratio
IS	=	Internal standard
LoDs	=	Limits of detection
PE	=	Polyethylene
SF-ICP-MS	=	Sector field inductively coupled plasma mass spectrometry

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