No Evidence of Genotoxic Damage in a Group of Patients with Titanium Dental Implants and Different Metal Restorations in the Oral Cavity

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ABSTRACT

Background: Titanium is the most widely used metal in implant dentistry. In spite of its biocompatibility, when it is released into the oral environment, it can have local negative biological effects.

Purpose: The aims of this study were to detect the concentration of metal ions in patients with dental implants, to evaluate whether or not their release might be influenced by the presence of other metals, and to assay whether these ions might provoke genotoxic damage in oral mucosa cells.

Materials and Methods: One hundred five patients with a total of 180 dental implants were included. The sample was divided into seven groups ($n = 15$ per group). Group 1 consisted of patients with metal-porcelain fixed crowns on dental implants; Group 2, patients with metal-porcelain fixed crowns on teeth; Group 3, patients with dental amalgams; Group 4, patients with metal-porcelain fixed crowns on dental implants and metal-porcelain fixed crowns on teeth; Group 5, patients with metal-porcelain fixed crowns on dental implants and dental amalgams; and Group 6, patients with metal-porcelain fixed crowns on dental implants, metal-porcelain fixed crowns on teeth, and dental amalgams. Group 7 was the control group, without any dental treatment. The concentration of metal ions was detected using inductively coupled plasma mass spectrometry; genotoxicity was measured using the buccal micronucleus cytome assay protocol.

Results: Group 5 displayed the highest concentration of metal ions in parts per billion (Ti, Co, Ni, Zn, Pd, Sn, and Pb). Group 6 was characterized by the highest presence of Hg. No signs of genotoxic damage were found in any of the study groups.

Conclusions: Patients with titanium dental implants combined with other metal restorations presented higher concentrations of metal ions, but no genotoxic damage was observed in oral mucosal epithelial cells.

KEY WORDS: dental implants, genotoxic damage, metal ions

INTRODUCTION

Titanium and its alloys are widely used in dentistry and orthopedics due to their biocompatibility, good mechanical properties, and resistance to corrosion.[1] When titanium is exposed to air or liquids, a layer of titanium oxide (TiO_2) forms that renders the metal unreactive. However, when titanium enters into contact with organic tissues and fluid, this initiates an electrochemical process that makes this material susceptible to fracture, which will release ions or metal particles into the oral environment.[2] The most widely used metals in implant dentistry and dental prosthetics are cobalt, chromium, nickel, titanium, aluminum, and vanadium. In vivo, corrosion of the metals used in the oral cavity causes two types of problem: (1) reduction of the material's mechanical properties and lifespan; (2) damaging local and systemic reactions by the host organism.

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In this way metal ions are released from implants as a result of corrosion,[3] and when other metals used in the oral cavity for a diverse range of restoration types coexist in the mouth, there will be a significant increase in the risk of ion release as a result of corrosion.[4] The literature describes various biological effects that can appear. These include hypersensitivity resulting from exposure to Ti particles released from the implant[5] and bone involvement resulting from the fact that Ti particles can accumulate in bone tissue. This may have adverse effects on the receptor activator of nuclear factor κB ligand and on osteoprotegerin, essential for osteoclast activation, suppressing osteoblasts, which may provoke bone resorption.[6] Titanium can also increase the sensitivity of the gingival epithelial cells to microorganisms, provoking an increase in monocyte infiltration and inflammation.[7] The literature also describes cases of cancer around dental implants.[8,9] It should be stressed that TiO_2 has been classified by the International Agency for Cancer Research as a possible carcinogen in humans (Group 2B).[10]

Heavy metals can interact directly with DNA, causing damage. The effects produced are increased inflammatory response, inhibition of cellular antioxidant mechanisms, increased lipid peroxidation, and inhibition of DNA repair; all these can contribute to or favor DNA mutations.[11] Nevertheless, several studies have shown the absence of any adverse effects on tissues and organs caused by titanium, its alloys, and other heavy metals.[12,13]

The oral mucosa provides a physical barrier to noxious substances that could be metabolized to generate cytotoxic products or potentially reactive mutagenics.[14] An accumulation of genomic damage leads to genetic instability, which may manifest as chromosomal disorders.

Cytogenetic biomonitoring is a minimally invasive method for examining biomarkers of DNA damage, chromosomal instability, and cell death in the oral mucosal epithelial cells. The oral mucosa can be used to detect early genotoxic events in patients or persons exposed to noxious agents. It is quick, sensitive, economic, and widely used as a reliable biomarker.[15]

The aim of the present study was to detect the concentration of metal ions in patients with dental implants and evaluate whether or not their release might be influenced by the presence or absence of other metals in the oral cavity and to assay whether these ions might provoke genotoxic damage in exfoliated oral mucosa cells.

MATERIALS AND METHODS

Recruitment and Patient Characteristics

This case-control study was carried out with a total of 105 patients (50 men and 55 women), aged between 30 and 54 years.

Inclusion criteria were age between 30 and 60 years and treatment with dental implants (with a minimum time of 2 months from prosthetic rehabilitation); presence or absence of other metal restorations in the oral cavity was noted.

Exclusion criteria were presence of peri-implant disease, dental treatment during the previous 6 months, exposure to ionizing radiation during the previous 6 months, taking antioxidant dietary supplements during the previous 6 months, and being younger than 30 or older than 60.

All patients gave their informed consent in writing. The study protocol was approved by the University of Murcia Ethics Committee and was carried out between September 2010 and December 2012 at two centers: the University Dental Clinic (University of Murcia, Murcia, Spain) and a private dental clinic (in the city of Murcia, Spain). The 105 patients were divided into seven groups ($n = 15$ per group). Group 1 consisted of patients with metal-porcelain fixed crowns on dental implants; Group 2 included patients with metal-porcelain fixed crowns on teeth; Group 3 contained patients with dental amalgams; Group 4 was composed of patients with metal-porcelain fixed crowns on dental implants and metal-porcelain fixed crowns on teeth; Group 5 comprised patients with metal-porcelain fixed crowns on dental implants and dental amalgams; Group 6 patients had metal-porcelain fixed crowns on dental implants, metal-porcelain fixed crowns on teeth, and dental amalgams; and Group 7 was the control group, without dental treatments.

Cell Sampling and Preparation

Exfoliated buccal cells were collected from each subject by a single practitioner. Prior to buccal cell collection, the mouth was rinsed with water to remove saliva, food particles, and any other debris. The insides of both cheeks were brushed using conventional toothbrushes in a circular motion 20 times. 30 ml yellow-top containers

were prepared containing 10 ml of buccal cell buffer (0.1 M EDTA, 0.01 M Tris-HCl, 0.02 M NaCl; pH = 7; E6758, Sigma-Aldrich, St. Louis, MO, USA). The brushes were placed into their respective buffer containers and rotated repeatedly to dislodge the cells and release them into the buffer. The cells were then transferred into centrifuge tubes and centrifuged for 10 minutes at 2,000 rpm ($581 \times g$). After centrifuging, the supernatant was aspirated, and cells were resuspended in another 5 ml of buccal buffer and centrifuged again; the process was repeated one further time.

In a separate tube, 100 µl of this cell suspension was treated with 0.5% nitric acid (2 ml) and the obtained cell lysate brought to 10 ml with ultrapure water (EASYpure II, Barnstead International, Boston, MA, USA) for metal ion quantification.

The samples were kept at −80°C until all the samples were collected.

Concentration of Metal Ions

The concentrations of the different metal ions (parts per billion = µg/L) were measured using inductively coupled plasma mass spectrometry (ICP-MS). The metal ions the concentrations of which were determined were Al^{27}, Ti^{47}, V^{51}, Cr^{52}, Mn^{55}, Fe^{56}, Co^{59}, Ni^{60}, Cu^{63}, Zn^{66}, Pd^{105}, Ag^{107}, Sn^{118}, Pt^{195}, Au^{197}, Pb^{208} and Hg^{200}.

Genotoxic Damage Study Using the Buccal Micronucleus Cytome Assay

Cells were transferred using a pipette, dropping 120 to 150 ml of cell suspension onto two clean and labeled microscope slides; after drying, these were placed in an oven at 55°C for 15 minutes and then fixed with 50% methanol (E-08211, Panreac S.A.U., Barcelona, Spain) at 0°C for 15 minutes and stained with DAPI® (4′,6-diamidino-2-phenylindole dihydrochloride; D9542, Sigma-Aldrich) at a concentration of 200 µg/ml, DAPI being a fluorescent dye that binds strongly to DNA. All slides were then washed in Milli-Q water (Milli Iberica, Madrid, Spain). The slides were scored using a Leica DRMB fluorescence microscope (Leica Microsystems, Wetzlar, Germany) equipped with a DAPI band filter (wavelength excitation filter set BP 340–380, dichroic filter RKP 400, and emission filter LP 425) at ×100 magnification.

Buccal micronucleus cytome assay (BMNcyt) scoring criteria for the various different cell types and/or nuclear anomalies were based on those prescribed by Tolbert and colleagues.[16] The purpose of these criteria was to classify buccal cells into categories that distinguish between normal cells and cells that are considered abnormal on the basis of cytological and nuclear features indicative of DNA damage: cytokinetic defects, proliferative potential, and cell death.

A more detailed description of the scoring criteria for BMNcyt assay cell types follows.

Cells with Micronuclei. Cells with micronuclei are characterized by the presence of both a main nucleus and one or more smaller nuclear structures called micronuclei. The micronuclei are round or oval in shape, and their diameter should range between one-third and one-sixteenth of the main nucleus. Micronuclei have the same staining intensity and texture as the main nucleus. Most cells with micronuclei will contain only one micronucleus, but it is possible to find cells with two or more micronuclei. The nuclei in micronucleated cells have the morphology of nuclei in normal cells. The micronuclei must be located within the cytoplasm of the cells. Micronuclei are scored only in differentiated cells with uniformly stained nuclei. It is possible to score micronuclei in basal cells, but this is impractical owing to the low frequency of this cell type.

Cells with Nuclear Buds. Cells with nuclear buds contain nuclei with an apparent sharp constriction at one end of the nucleus, suggestive of a budding process and elimination of nuclear material by budding. The nuclear bud and the nucleus are usually in very close proximity and appear to be attached to each other. The nuclear bud has the same morphology and staining properties as the nucleus; however, its diameter may range from a half to a quarter of that of the main nucleus.

Binucleated Cells. Binucleated cells are cells containing two main nuclei instead of one. The nuclei are usually very close and may touch each other and usually have the same morphology as that observed in normal cells.

Basal Cells. Basal cells have a larger nucleus-to-cytoplasm ratio than differentiated buccal cells. Basal cells have a uniformly stained nucleus and are smaller in size and more oval in shape when compared to the more angular and flat differentiated buccal cells.

Differentiated Cells. Normal differentiated cells have a uniformly stained nucleus, which is oval or round in

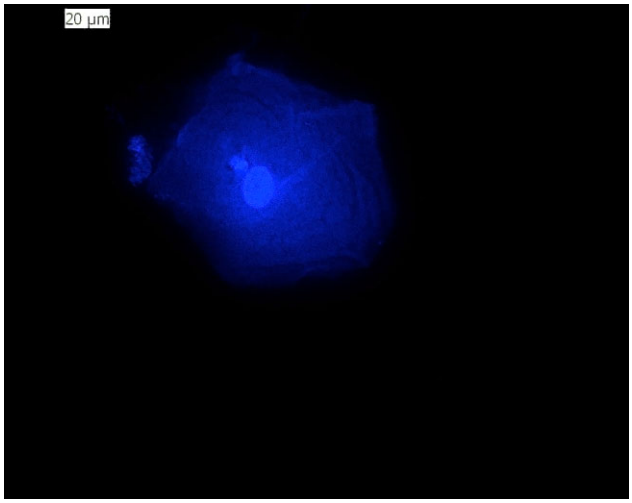

Figure 1 Differentiated cell with micronucleus (×100).

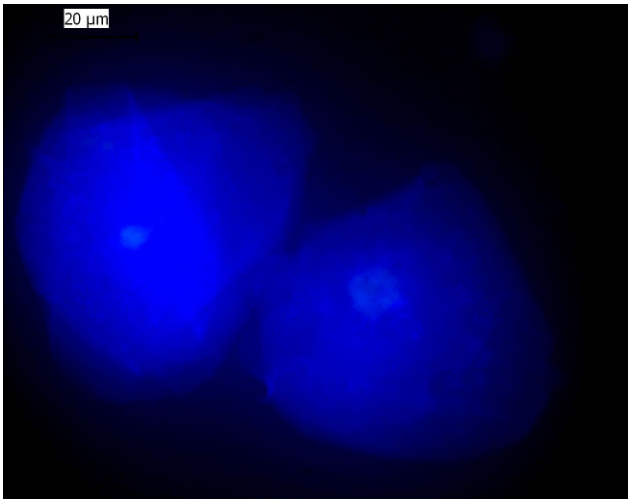

Figure 2 Pyknotic cell (left) and cell with karyorrhexis (right) (×100).

shape. They are distinguished from basal cells by their larger size and by a smaller nucleus-to-cytoplasm ratio (Figure 1).

Condensed Chromatin. Buccal cells with condensed chromatin show a roughly striated nuclear pattern in which the aggregated chromatin is intensely stained. In these cells it is apparent that chromatin is aggregating in some regions of the nucleus while being lost in other areas. When chromatin aggregation is extensive, the nucleus may appear to be fragmenting.

Karyorrhectic Cells. Karyorrhectic cells have nuclei that are characterized by more extensive nuclear chromatin aggregation relative to cells with condensed chromatin. They have a densely speckled nuclear pattern, indicative of nuclear fragmentation that will lead to the eventual disintegration of the nucleus (Figure 2).

Pyknotic Cells. Pyknotic cells are characterized by small shrunken nuclei, with a high density of nuclear material that is uniformly but intensely stained. The nuclear diameter is usually one- to two-thirds of the nuclei of normal differentiated cells (Figure 2).

Karyolytic Cells. Karyolytic cells are cells in which the nucleus is completely depleted of DNA and is apparent as a ghostlike image (Figure 3).

Scoring Method. Initially, the buccal cytome assay scored 1,000 cells per subject for the various cells types: those containing basal cells, binucleates, and cell death parameters (condensed chromatin cells, karyorrhectic cells, pyknotic cells, and karyolytic cells). Micronuclei and nuclear buds were scored over 2,000 cells. Only basal and normal differentiated cells were scored for micronuclei, and their scores were combined to give the overall incidence.

Statistical Analysis

Data were analyzed using SPSS® 12.0 statistical software (SPSS Inc., Chicago, IL, USA). A descriptive study was made of each variable. The Kolmogorov-Smirnov normality test and the Levene variance homogeneity test were applied; the data showed a skewed distribution and so were analyzed using a nonparametric ranking test.

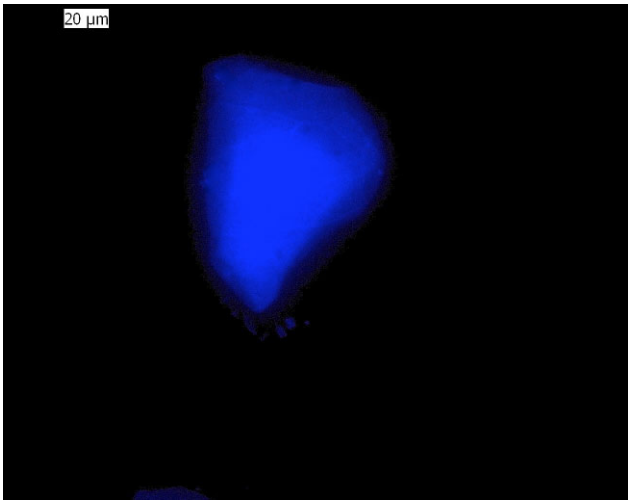

Figure 3 Cell with karyolysis (×100).

The associations between the different qualitative variables were studied using Pearson's chi-square test. The Kruskal-Wallis test for more than two samples was used for quantitative variables. Probability of less than 5% ($p < .05$) was accepted as significant.

RESULTS

The study examined a sample of 105 patients (50 men and 55 women), with an average age of 38 years (ranging between 30 and 54 years). 70.5% of the sample were nonsmokers, and 45.7% did not drink alcohol. The majority did not suffer any systemic disease and presented an average number of teeth (27, range 10–32) (Table 1).

Table 2 shows the numbers of different types of metallic restoration among the sample group and the average times (in months) they had been present in patients' mouths. The most frequently occurring metal restorations were metal-porcelain crowns on dental implants ($n = 182$). Dental amalgam was the restoration type with the longest time in the mouth, an average of 138 months (range 26–276 months).

Table 3 describes the characteristics of the 180 dental implants placed in the 105 patients who made up the sample. Most of the implants (53.9%) were placed in the maxilla, and 46.1% were placed in the mandible; the most frequently used implant length was 10 mm (27.2%), while the most frequent implant diameter was 4.5 mm (61.1%).

The seven study groups were homogeneous with regard to age ($p = .069$), sex ($p = .993$), smoking ($p = .795$), alcohol consumption ($p = .678$), and body mass ($p = .091$) (Table 4).

Table 5 shows the concentrations of metal ions detected by ICP-MS in saliva samples. Ti concentrations in saliva were practically nil in all study groups, except for Group 5 (metal-porcelain fixed crowns on dental implants + dental amalgams; median 1.02, range 0.00–1.02) and Group 6 (metal-porcelain fixed crowns on dental implants + metal-porcelain fixed crowns on teeth + dental amalgams; median 0.89, range 0.00–0.89). The highest concentration of Ni was found in Group 6 (metal-porcelain fixed crowns on dental implants + metal-porcelain fixed crowns on teeth + dental amalgams; median 3.18, range 1.23–6.52), with a statistically significant difference ($p = .037$). Group 1 (metal-porcelain fixed crowns on dental implants) presented the highest concentration of Cu (median 23.63, range

TABLE 1 Characteristics of the Study Population

Patients, *n*	105
Age, median (range)	38 (30–54)
Sex, *n* (%)	
Male	50 (47.62)
Female	55 (52.38)
Educational level, *n* (%)	
None	2 (1.92)
Primary	15 (14.28)
Secondary	35 (33.33)
University	53 (50.47)
Body mass index (kg/m^2), median (range)	25.49 (18.68–33.89)
Smoking status, *n* (%)	
Nonsmoker	74 (70.47)
<10 cigarettes/day	15 (14.28)
11–20 cigarettes/day	11 (10.47)
>20 cigarettes/day	5 (4.78)
Alcohol consumption, *n* (%)	
None	48 (45.72)
Daily	0 (0)
Weekend drinker	57 (54.28)
Diseases, *n* (%)	
Cardiovascular disease	6 (5.71)
Endocrine disease	9 (8.57)
Neurological disease	3 (2,85)
Respiratory disease	2 (1.92)
Locomotor deficit	1 (0.95)
Gastrointestinal disease	9 (8.57)
Rheumatologic disease	2 (1.92)
Number of teeth, median (range)	27 (10–32)
Dental treatments, *n* (%)	
M-P fixed crowns on DI (Group 1)	15 (14.28)
M-P fixed crowns on teeth (Group 2)	15 (14.28)
Dental amalgams (Group 3)	15 (14.28)
M-P fixed crowns on DI + M-P fixed crowns on teeth (Group 4)	15 (14.28)
M-P fixed crowns on DI + dental amalgams (Group 5)	15 (14.28)
M-P fixed crowns on DI + M-P fixed crowns on teeth + dental amalgams (Group 6)	15 (14.28)
None (Group 7)	15 (14.28)
Metallic occlusion: *n* (%)	
Yes	28 (26.67)
No	77 (73.33)

M-P, metal-porcelain; DI, dental implants.

TABLE 2 Metallic Dental Treatments in the Study Population

Characteristic	*n*	Time Placed in Mouth or Time of Metallic Occlusion (Months), Median (Range)
Dental treatments		
M-P fixed crowns on DI	182	48 (2–96)
M-P fixed crowns on teeth	131	72 (4–240)
Dental amalgams	154	138 (26–276)

M-P, metal-porcelain; DI, dental implants.

TABLE 3 Implant Distribution

Characteristic	*n* (%)
Number of dental implants	180 (100)
Dental implant type	
Brånemark System (Nobel Biocare® Ibérica S.A., Barcelona, Spain)	6 (3.35)
Biotech® (Biotech International, Marseille, France)	35 (19.44)
Klockner® (Klockner Implant System S.A., Barcelona, Spain)	36 (20)
Biomet 3i® (Biomet 3i Dental Ibérica S.L., Barcelona, Spain)	63 (35)
Phibo® (Phibo, Barcelona, Spain)	11 (6.11)
Avinent® (Avinet Implant System, Barcelona, Spain)	13 (7.22)
Zimmer® (Zimmer Dental Inc., Barcelona, Spain)	16 (8.88)
Maxilla/mandible, *n* (%)	
Maxilla	97 (53.88)
Mandible	83 (46.12)
Anterior/posterior, *n* (%)	
Anterior	19 (10.56)
Posterior	161 (89.44)
Length (mm), *n* (%)	
10	49 (27.22)
11.5	34 (18.88)
13	35 (19.44)
14.5	35 (19.44)
16	27 (15.02)
Diameter (mm), *n* (%)	
3.3	15 (8.34)
3.5	55 (30.55)
4.5	110 (61.11)

TABLE 4 Homogeneity of the Study Groups in Terms of Demographic Characteristics, Habits, and Body Mass Index

Characteristic	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	*p* Value
Age, median (range)	37.00 (35.00–50.00)	38.00 (34.00–39.00)	37.00 (35.00–41.00)	39.00 (35.00–54.00)	39.00 (35.00–39.00)	37.00 (35.00–45.00)	39.00 (30.00–48.00)	.069
Sex, *n* (%)								.993
Male	8 (53.33)	7 (46.67)	8 (53.33)	7 (46.67)	7 (46.67)	7 (46.67)	6 (40.00)	
Female	7 (46.66)	8 (53.33)	7 (46.67)	8 (53.33)	8 (53.33)	8 (53.33)	9 (60.00)	
Smoking, *n* (%)								.795
Yes	2 (13.34)	4 (26.67)	5 (33.33)	4 (26.67)	5 (33.33)	5 (33.33)	6 (40.00)	
No	13 (86.66)	11 (73.33)	10 (66.67)	11 (73.33)	10 (66.67)	10 (66.67)	9 (60.00)	
Alcohol consumption, *n* (%)								.678
Yes	6 (40.00)	9 (60.00)	10 (66.67)	7 (46.67)	8 (53.33)	10 (66.67)	7 (46.67)	
No	9 (60.00)	6 (40.00)	5 (33.33)	8 (53.33)	7 (46.67)	5 (33.33)	8 (53.33)	
Body mass index (kg/m^2), median (range)	28.38 (19.48–33.19)	24.51 (21.99–26.11)	21.92 (20.61–28.77)	26.71 (21.24–29.59)	25.95 (25.49–29.49)	24.25 (18.68–29.68)	24.85 (20.31–33.89)	.091

n = 15 per group.
p Values determined by Kruskal-Wallis test and Pearson's chi-square test.
Group 1, metal-porcelain fixed crowns on dental implants; Group 2, metal-porcelain fixed crowns on teeth; Group 3, dental amalgams; Group 4, metal-porcelain fixed crowns on dental implants + metal-porcelain fixed crowns on teeth; Group 5, metal-porcelain fixed crowns on dental implants + dental amalgams; Group 6, metal-porcelain fixed crowns on dental implants + metal-porcelain fixed crowns on teeth + dental amalgams; Group 7, control group without dental treatments.

TABLE 5 Concentrations of Metal Ions (ppb) Detected in Salivary Secretion by Study Group

Metal Ion	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	*p* Value
Al^{27}	22.16 (16.73–170.39)	20.61 (2.76–37.76)	14.77 (5.14–24.61)	18.68 (6.24–70.68)	20.85 (17.77–20.85)	19.46 (1.65–44.86)	7.51 (2.07–14.57)	<.001
Ti^{47}	0.00 (0.00–1.39)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–2.42)	1.02 (0.00–1.02)	0.89 (0.00–0.89)	0.00 (0.00–0.00)	<.001
V^{51}	0.25 (0.09–0.29)	0.24 (0.06–0.25)	0.27 (0.11–0.33)	0.24 (0.09–0.49)	0.21 (0.21–0.42)	0.21 (0.00–0.34)	0.13 (0.07–0.22)	.002
Cr^{52}	1.04 (0.42–4.98)	0.91 (0.27–1.25)	1.25 (0.47–1.27)	0.97 (0.41–2.88)	1.03 (1.01–1.03)	1.21 (0.18–1.66)	0.41 (0.28–0.96)	.003
Mn^{55}	2.71 (1.23–5.11)	1.48 (0.62–5.63)	5.23 (0.93–6.31)	3.08 (1.22–4.06)	3.82 (3.31–3.82)	3.71 (0.29–5.21)	1.31 (1.12–1.43)	<.001
Fe^{56}	23.72 (8.47–244.97)	14.47 (3.94–25.26)	17.73 (6.32–24.84)	26.86 (8.56–172.39)	37.77 (37.33–57.33)	23.07 (3.61–32.07)	5.51 (3.72–12.95)	<.001
Co^{59}	0.61 (0.24–0.95)	0.22 (0.13–0.87)	0.19 (0.18–0.83)	0.63 (0.22–1.86)	0.76 (0.53–0.76)	0.76 (0.16–0.89)	0.24 (0.23–0.25)	.003
Ni^{60}	2.48 (0.53–5.21)	2.04 (0.66–4.72)	1.85 (0.63–2.01)	1.53 (0.83–4.32)	1.77 (0.44–2.99)	3.18 (1.23–6.52)	1.36 (1.36–1.61)	.037
Cu^{63}	23.63 (7.38–48.93)	10.63 (2.81–23.36)	7.07 (6.56–10.84)	7.34 (3.38–221.33)	10.56 (2.54–21.11)	11.31 (3.31–19.39)	6.79 (5.46–6.79)	<.001
Zn^{66}	126.57 (36.36–788.67)	36.52 (14.98–102.02)	151.46 (45.71–176.16)	113.49 (17.73–400.59)	195.51 (106.86–195.51)	115.49 (11.47–207.11)	28.24 (16.87–29.38)	<.001
Pd^{105}	0.11 (0.00–0.87)	0.32 (0.21–0.52)	0.16 (0.12–0.35)	0.41 (0.11–1.72)	0.87 (0.67–0.87)	0.17 (0.00–0.47)	0.11 (0.00–0.42)	<.001
Ag^{107}	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.23)	0.00 (0.00–0.00)	.423
Sn^{118}	0.63 (0.00–4.36)	0.00 (0.00–2.01)	0.00 (0.00–3.58)	1.71 (0.00–8.12)	3.25 (2.71–3.25)	0.97 (0.00–1.44)	0.97 (0.00–1.44)	<.001
Pt^{195}	0.00 (0.00–0.71)	0.00 (0.00–0.06)	0.00 (0.00–0.00)	0.04 (0.00–0.14)	0.06 (0.04–0.06)	0.00 (0.00–0.00)	0.00 (0.00–0.04)	<.001
Au^{197}	0.00 (0.00–0.18)	0.00 (0.00–4.51)	0.00 (0.00–0.00)	0.18 (0.00–2.16)	1.09 (0.58–1.09)	0.00 (0.00–0.00)	0.00 (0.00–0.15)	.061
Pb^{208}	1.32 (0.74–25.36)	0.31 (0.00–2.77)	0.00 (0.00–1.34)	1.77 (0.00–9.72)	2.47 (2.47–7.03)	0.89 (0.00–4.62)	0.00 (0.00–0.00)	<.001
Hg^{200}	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.00 (0.00–0.48)	0.00 (0.00–0.00)	0.67 (0.00–8.82)	2.39 (1.05–2.39)	0.00 (0.00–0.00)	<.001

$n = 15$ per group.
Values for groups given as median (range).
p Values determined by Kruskal-Wallis test.
Group 1, metal-porcelain fixed crowns on dental implants; Group 2, metal-porcelain fixed crowns on teeth; Group 3, dental amalgams; Group 4, metal-porcelain fixed crowns on dental implants + metal-porcelain fixed crowns on teeth; Group 5, metal-porcelain fixed crowns on dental implants + dental amalgams; Group 6, metal-porcelain fixed crowns on dental implants + metal-porcelain fixed crowns on teeth + dental amalgams; Group 7, control group without dental treatments.

7.38–48.93), with a statistically significant difference ($p < .001$).

Overall, Group 5 displayed the highest concentration of metal ions (parts per billion; Ti, Co, Ni, Zn, Pd, Sn, and Pb), followed by Group 3 (V, Cr, and Mn). Group 6 was characterized by the highest presence of Hg (median 2.39, range 1.05–2.39), with a statistically significant difference ($p < .001$).

With regard to genotoxicity, Group 5 (metal-porcelain fixed crowns on dental implants + dental amalgams) presented the highest levels of condensed chromatin (median 63, range 50–63), with a statistically significant difference ($p = .004$). However, no signs of genotoxic damage were found in any of the study groups (Table 6).

DISCUSSION

The present study investigated whether patients with dental implants supporting porcelain-metal prosthetics in the presence of other metal restorations show greater ion release resulting from corrosion and, if so, how these metal ions might affect DNA and the regenerative capacity and apoptosis of the oral mucosal epithelial cells. Due to its excellent mechanical, physical, and chemical qualities, titanium and its alloys exhibit high resistance to corrosion and good biocompatibility in a physiological environment.[17] However, most metals in contact with a biological medium do suffer corrosion, leading to the release of metal ions.[2] Although titanium and its alloys are known for their high resistance to corrosion, the possibility that some degree of corrosion might be produced in the biological medium should not be ignored.[18] Furthermore, even though a metal may be resistant to corrosion by itself, when it is placed in the medium alongside other metals, the risk of corrosion will be considerably increased.[4]

Nevertheless, in light of the present study, it must be stressed that as a highly corrosion-resistant element, titanium was present in study Groups 1, 4, 5, and 6, but the concentration of ions in saliva was 0 ppb in Groups 1 and 4 and very low in Groups 5 and 6. In the two latter groups, the patients also had implant-supported metal-porcelain crowns as well as silver amalgam. In Group 6 (metal-porcelain fixed crowns on dental implants + metal-porcelain fixed crowns on teeth + dental amalgams), higher levels of Hg were released than in Group 3 (dental amalgams) and Group 5 (metal-porcelain fixed crowns on dental implants + dental amalgams). This finding does not correspond to the results obtained by Shi-Duk and colleagues,[4] who observed that mercury ion release decreased when amalgams were in contact with titanium. They also found that copper ion release increased as a result of corrosion in these same cases. It could be that in the present study it was the silver amalgam that provoked greater metal ion release both from the implant surfaces and the metal restorations in Groups 5 and 6 than in the rest of the groups.

In general, Group 5 showed the highest concentrations of metal ions (Ti, Co, Ni, Zn, Pd, Sn, and Pb). To date, there has been no research into the toxicity of metal ions in patients with dental implants with or without the presence of other metals in the oral cavity. With regard to possible adverse biological effects of Ti and other metals placed in the oral cavity, Javed and colleagues[19] made a literature review on whether sensitivity to titanium could be related to allergic reactions to this metal; they found only seven articles and were unable to resolve the issue.

In vitro studies such as that of Makihira and colleagues[7] that examined Ti toxicity on gingival epithelial cells, cultivated to evaluate gingival tissue response to different concentrations of Ti ions, concluded that Ti might be involved in cytotoxic and inflammatory phenomena in the gingival tissue surrounding implants.

Sun and colleagues[20] evaluated Al, Co, Cr, Ni, Ti, and V ion release from dental implants at different concentrations to see how they might affect osteoblast metabolism and differentiation. The results showed that metal ion release can alter osteoblastic behavior even at subtoxic levels.

In spite of the ion release observed in the present study in patients with titanium dental implants together with other metal restorations, no indications of genotype damage were found in the epithelial cells scraped from the oral mucosa. The frequencies of micronuclei, nuclear buds, and binucleated cells, considered to be biomarkers of genotype damage, were similar in all study groups and without statistically significant difference.

The cytogenetic biomonitoring assay used in the present study, named the BMNcyt assay by Thomas and colleagues,[21] is a minimally invasive method for studying damage to DNA, chromosome instability, cell death, and the regenerative potential of oral mucosa tissues. This method is being used with increasing frequency to

TABLE 6 DNA Damage (Cytokinetic Defects, Proliferative Potential, and Cell Death) by Study Group

Cytogenetic Variable	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	*p* Value
Cytokinetic defects								
Micronuclei	2 (0–4)	2 (0–4)	1 (1–4)	2 (1–4)	1 (1–4)	1 (1–4)	2 (1–3)	.556
Nuclear buds	0 (0–2)	1 (0–1)	1 (0–2)	0 (0–1)	1 (1–2)	0 (0–1)	0 (0–1)	.433
Binucleated cells	3 (0–10)	3 (1–9)	2 (1–3)	4 (1–10)	1 (1–7)	3 (3–4)	3 (0–11)	.355
Proliferative potential								
Basal cells	15 (7–45)	21 (9–51)	15 (10–53)	17 (7–51)	19 (9–49)	17 (17–52)	17 (7–48)	.366
Differentiated cells	650 (620–809)	650 (620–715)	620 (66–711)	666 (632–902)	743 (700–743)	620 (620–755)	610 (610–800)	.063
Cell death								
Condensed chromatin	41 (21–56)	53 (52–58)	44 (42–63)	53 (40–57)	63 (50–63)	51 (39–55)	40 (34–67)	.004
Karyorrhectic cells	17 (5–33)	29 (10–42)	26 (2–40)	15 (10–20)	17 (13–17)	30 (11–30)	20 (8–47)	.534
Pyknotic cells	2 (0–5)	2 (1–4)	1 (!–2)	2 (2–6)	2 (4–7)	3 (1–4)	3 (1–15)	.623
Karyolytic cells	70 (50–90)	76 (72–81)	70 (57–61)	69 (50–90)	72 (60–72)	67 (50–70)	63 (50–78)	.075

$n = 15$ per group.
Values for groups given as median (range).
p Values determined by Kruskal-Wallis test.
Group 1, metal-porcelain fixed crowns on dental implants; Group 2, metal-porcelain fixed crowns on teeth; Group 3, dental amalgams; Group 4, metal-porcelain fixed crowns on dental implants + metal-porcelain fixed crowns on teeth; Group 5, metal-porcelain fixed crowns on dental implants + dental amalgams; Group 6, metal-porcelain fixed crowns on dental implants + metal-porcelain fixed crowns on teeth + dental amalgams; Group 7, control group without dental treatments.

research the impact of nutrition, ionizing radiation, chemical agents, environmental contaminants, life style, and genotoxic exposure in general.[22]

There are several studies of genotoxic damage in the mouth, such as that by Faccioni and colleagues,[23] that showed that subtoxic concentrations of metals can be sufficient to provoke biological effects in oral mucosa cells; the metals can provoke pathological effects such as increased inflammatory responses, inhibition of antioxidant defenses, increased lipid peroxidation, and inhibition of DNA repair.[11] Other studies, like the present one, have failed to find any genotoxic damage in the organism from metals such as titanium. For example, Piozzi and colleagues[24] studied cytotoxicity and genotoxic damage from titanium and its alloys using miniplates in vivo in sensitive organs, such as the lungs, kidneys, and livers, of laboratory rats. They found that the titanium miniplates did not provoke DNA damage to these organs. Matsumoto and colleagues[25] made in vitro observations of two cell lines (fibroblasts and osteoblasts), finding that dental implants undergoing corrosion did not cause DNA damage (evaluated by comet assay). Another study done by the same research team on dental implants undergoing corrosion failed to detect DNA strand breaks in in vitro Chinese hamster ovary cells.[26]

Nevertheless, the majority of published research claims that titanium and other metals are agents with probable genotoxic and cytotoxic activity in humans. Some even state that subtoxic concentrations of some metals may be sufficient to provoke important biological effects in some cell systems. Experimental and epidemiological studies have shown how nickel composites may be associated with nasal or lung cancer, that cobalt may also be a carcinogenic agent,[27] and that some metals could act as cogenotoxicants, provoking DNA damage (oxidative damage and interference in DNA repair and replication systems).[28] Cases of cancer around dental implants have also been described[8,9] and there are 14 studies in the literature that describe carcinomas around dental implants, most of which were oral squamous cell carcinomas.[29] However, the carcinogenic mechanisms have not been established and the association between titanium particles and cancer development has not been demonstrated.

To date, no other study in the literature has studied genotoxic damage in squamous epithelial cells in the oral cavity due to the action of metal ions released in patients with titanium dental implants as well as other metal dental restorations.

The present study found that the concentration of some metal ions, such as Ti^{47}, in patients with metal-porcelain fixed crowns on dental implants was not greater than in the control group (without dental treatments). However, the release of metal ions in patients with a combination of metal-porcelain fixed crowns on dental implants and other metals used for prosthetics and dental restorations, in particular silver amalgams, was greater than in patients who did not have this combination of metals in the oral cavity. Nevertheless, these did not provoke any DNA damage, nor did they affect the regenerative potential or cell death of oral mucosa epithelial cells. No evidence was discovered for the genotoxic effects of titanium or its alloys or other metals employed for restoration in the oral cavity. Dental implants, whether in combination with other metal restorations or not, are innocuous and so ideal for the esthetic and functional rehabilitation of partially or totally edentulous patients.

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