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Salivary metal levels of orthodontic patients: a novel methodological and analytical approach

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SUMMARY The purpose of this study was to qualitatively and quantitatively assess the nickel, chromium, and ferrous levels in a population of 17 orthodontic patients undergoing treatment, compared with seven untreated individuals, employing a novel methodological approach and a new analytical technique. Salivary samples obtained from patients before and after rinsing with double distilled water were processed for Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) for simultaneous estimation of the concentration of the metallic elements.

No statistically significant difference was detected between control and patient groups with respect to salivary metal content, regardless of element. The range of salivary metal levels found did not exceed those of daily intake through food and air.

The lack of a continuous monitoring scheme for salivary metal concentrations in real time may impose substantial obstacles to defining the release rate of metals *in vivo.* The results of this study emphasize the necessity of incorporating the dimension of time in assessing the release potential of orthodontic alloys.

Introduction

The issue of biomaterial-derived ionic release in various sites of the human body has attracted the interest of many investigators because of the possibility that debris or degradation products elicit a foreign body reaction, or have a role in the induction of pathological processes (Jasty *et al.*, 1992; Lee *et al.*, 1995; Jones *et al.*, 2001). Thus, research in the broader biomedical literature has focused on the release of substances from various devices including orthopaedic (Cook *et al.*, 1985; Lewis and Sunderman, 1996) and dental materials. The latter category mainly comprises orthodontic (Gjerdet *et al.*, 1991; Locci *et al.*, 2000; Tomakidi *et al.*, 2000), restorative (Olea *et al.*, 1996), and prosthetic materials (Lygre *et al.*, 1995), which come into contact with biological tissues or fluids such as saliva or blood.

In orthodontics, major emphasis has been placed on the release of nickel or chromium (Kerosuo *et al.*, 1997; Jia *et al.*, 1999; Staffolani *et al.*, 1999) owing to the well established hazardous nature of those elements (Salnikow *et al.*, 2000). The American Iron and Steel Institute (AISI) type 316L austenitic stainless steel alloy currently used for bracket manufacturing contains 10–14 per cent nickel and 18–20 per cent chromium by weight. Nickel is incorporated in all austenitic stainless steel alloys to stabilize the austenite phase, improve the anti-corrosive property of the alloy, and decrease the ductility, whilst chromium is added to facilitate the formation of an anti-corrosive passive film (Brantley, 2001).

The harmful effects of elements such as nickel have long been systematically investigated at the cell, tissue, organ, and organism levels, and the sequence of the biological reactions associated with its presence has been illustrated (Costa and Hollenhauer, 1980). Nickel concentrations within the range of those found to be released from dental alloys may activate monocytes and endothelial cells, suppressing or promoting the expression of intercellular adhesion molecule 1 by endothelial cells, depending on ionic concentration (Wataha *et al.*, 1997, 1999a). In addition, nickel complexes in the form of arsenides and sulphides have long been known as carcinogenic, allergenic, and mutating substances, whereas nickel at non-toxic concentrations (Costa and Hollenhauer, 1980) has been found to induce DNA alterations mainly through base damage and DNA strand scission (single strand breaks), which are site specific. The mutative action of nickel may derive from its effect on inhibiting several enzymes known to restore DNA breaks (Kasprzak and Bialkowski, 2000), promoting microsatellite mutations, and increasing total genomic methylation, thereby contributing to genetic instability.

The hypothesis tested in this research was that orthodontic treatment induces an increase in the salivary metal content of patients. Therefore, the purpose of this study

was to assess qualitatively and quantitatively the salivary metal content of orthodontic patients.

Materials and methods

The salivary samples used in this study were collected from the practice of one author (EK) during regular visits of the patients. This pilot study included 17 subjects (nine boys and six girls) with a mean age of 16 years (range 13–18 years) and full bond appliances placed for at least 15 months. Seven age-matched individuals (four boys and three girls) with no intraoral appliances served as the controls. The selection of subjects was made randomly from a pool of 40 participants using the following criteria: no systemic disease or medication received *per os*, absence of restorations, and full bonding and banding in the maxillary and mandibular arches for at least 12 months.

All patients were treated with nominally standard procedures involving stainless steel brackets of 0.022-inch slot (Dentaurum, Pforzheim, Germany), which had not undergone any treatment such as recycling, and stainless steel archwires of the same brand, thus exposing the intraoral environment to nominally identical conditions. Patients were informed of the purpose of this study and consent was obtained. Each individual was assigned a code to facilitate an unbiased treatment sample, and saliva collection was performed during their visits at two intervals: before and after rinsing with double distilled water. Saliva samples weighing approximately 5 g were collected for each participant, placed in non-metal-containing plastic tubes, and frozen until processing.

The collected saliva samples were subjected to Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) with the use of an ICP-AES unit (Perkin Elmer, Optima 3000, USA). The technique is based on the principle that liquidized specimens of materials following treatment with acids and exposure to a temperature in the range of 5000–10 000 K emit light, which is recorded by multiple photodetectors (Svanberg, 1992). Calibration of the apparatus was achieved using standard solutions, and computer-controlled data collection was used to interpret the results. Under these conditions the detection threshold of the technique reaches levels as low as 1 ng/ml, or 1 part per billion (1 ppb).

For this study, briefly, approximately 10–15 ml of saliva was added in an open teflon vessel of 50 ml and the saliva sample was dried under heating with infrared radiation. Three millilitres of aqua regia was added to the dry product, the teflon vessel was closed, and the dry product was then dissolved under heating with infrared radiation. The homogenous solution obtained was diluted to a volume of 25 ml in a volumetric flask and the Ni, Fe, and Cr contents were determined using calibration curves. Calibration standards were made from Merck standard solutions and were formulated to be matrix-matched to the saline-contained samples.

The results were analysed statistically using a two-way ANOVA, with orthodontic treatment and rinsing serving as discriminating variables, and Tukey's multiple comparisons test at $\alpha = 0.05$ level of significance.

Results

The metal concentration values (ng/ml) for the Ni, Cr and ferrous (Fe) ions in the saliva of the two groups before and after rinsing with water are provided in Table 1. No statistically significant differences were found among groups with respect to metal concentrations. For some samples exact concentrations could not be estimated as they ranged below the threshold of the apparatus. The range of metal levels detected fell within that of daily intake through water and food.

Discussion

The results of the present investigation confirm previous findings reporting that the salivary metal levels of patients undergoing treatment do not exceed those of daily intake through food and air. The negative association of orthodontics with ionic release observed in this study may answer the recently increased demands for safety precautions for orthodontic patients set by various legislations (Turpin, 2001). Furthermore, the concentrations found are in the range of those traced in tap water or dietary supplements.

The majority of the evidence found in the literature is limited to *in vitro* approaches utilizing various immersion solutions of different acidity to examine the variation of the rate and total amount of ionic release (Barrett *et al*., 1993). The results of these studies cannot be applied to the clinical situation because of the apparent clinical irrelevance of the methodology used.

Table 1 Salivary metal content (ng/ml or ppb) of orthodontic patients and controls, before and after rinsing.

Group	Nickel Mean (SD)	Chromium Mean (SD)	Ferrous Mean (SD)	Tukey grouping
Controls before rinsing	18 (11)	20 (11)	21 (12)	A
Controls after rinsing	11 (7)	*	*	A
Patients before rinsing	*	*	14 (9)	A
Patients after rinsing	10 (6)	27 (11)	17 (14)	A

*Concentration below the 1 ppb threshold of instrumental analysis. Means with same letter (column comparisons) are not statistically different at $\alpha = 0.05$ level of significance.

On the other hand, the literature lists only a handful of papers investigating salivary metal levels in patients (Kerosuo *et al*., 1997; Gjerdet *et al*., 1999; Huang *et al*., 2001; Kocadereli *et al*., 2001). Those research efforts have focused mainly on nickel and chromium and employed exclusively atomic absorption to analyse the samples obtained from patients 1–2 months following bonding.

In general, *in vivo* investigations have indicated an increased salivary concentration of nickel and iron three weeks following the insertion of fixed orthodontic appliances. However, large individual variations deriving from the high variability in the number of bands and brackets of each participant precluded statistically significant differences in nickel concentration (Kerosuo *et al*., 1997). Recently published studies examining the nickel and chromium levels in saliva have not revealed an increased concentration of these ions one month after insertion of fixed appliances relative to the levels prior to insertion (Gjerdet *et al*., 1991; Staffolani *et al*., 1999; Kocadereli *et al*., 2001). These studies identified increased metal levels in the saliva of patients immediately following bonding, but reported no differences after three weeks.

It must be noted, however, that the foregoing investigations have focused on estimating the release occurring under specific conditions, which were far from being close to the routine clinical situation (Eliades and Athanasiou, 2002). A number of concerns have been raised pertinent to the methodology described in the majority of articles published on this issue (Eliades *et al*., 2002). The time periods employed for the *in vivo* ageing of materials were substantially low and the saliva sampling periods adopted did not exceed one month, a time interval almost 20 times shorter than the typical duration of treatment. As a result, the effect of corrosion processes and phenomena such as wear and fatigue on the release of nickel could not be elucidated. The latter is due to the fact that in multiple phase alloys, long-term release may be higher than that occurring within the first week, whereas single phase alloys present various release patterns with increasing or decreasing rates depending on the element released (Wataha *et al*., 1999b). Therefore, the results of studies employing time intervals within a one month range for the investigation of ionic release should be treated with scepticism.

Moreover, saliva sampling in those studies was performed momentarily, resulting in a notable lack of continuous and cumulative data extending over a wide time period. This unavoidable limitation derives from the nebulous nature of the examined variable, which does not permit continuous monitoring of salivary metal concentrations in real time.

Lastly, the protocol for saliva collection, which involves stimulation through chewing on a piece of paraffin wax or gum, inevitably restricts the collection of saliva to that secreted almost directly from the gland. This effect arises from the lack of saliva wetting of the oral cavity including teeth, limiting the exposure of appliances to salivary flow, and thus, possibly inducing a false negative result.

In an attempt to address these concerns, the current investigation utilized a different approach in selecting the patient population, obtaining salivary samples, and employing an alternative analytical approach to investigate their metal content. Firstly, the effect of ageing of metal components of appliances and wires was incorporated in the design of the study by including individuals who had been under treatment for more than a year. Secondly, salivary samples were obtained under two different conditions: immediately when patients entered the office, and following rinsing with double distilled water to assess the effect of rinsing in altering the release potential. The reason behind this regimen pertains to the possibility that rinsing may mask the salivary levels of metals since it dilutes the metal content. Finally, atomic emission versus atomic absorption spectroscopy was chosen for analysis based on the low threshold and capacity of providing levels of multiple metals simultaneously. Atomic emission is capable of providing information about a wide spectrum of elements in the same sample as opposed to atomic absorption where the investigations cannot be performed simultaneously.

Some investigators have followed a different approach in evaluating nickel leaching, examining the blood serum nickel level of orthodontic patients (Bishara *et al*., 1993). Blood serum and urine concentration for a metal, however, are dependent on highly individualized parameters such as clearance and excretory rates, which are species- and element-specific. For example, in rabbits, Ni shows a high affinity for kidneys, whilst molybdenum is selectively accumulated in the spleen (Black, 1984). Therefore, given the unknown clearance and excretory rate for nickel in humans, the observation that Ni levels in the blood of orthodontic patients are no different from those obtained from untreated individuals cannot exclude the possibility that Ni has been released and accumulated in an organ.

The results of the present study are in agreement with previous work (Gjerdet *et al.*, 1991) which failed to document increased levels of metal in the saliva of orthodontic patients. From a different perspective, however, the nature of the variable studied, involving investigation of a continuous release pattern through momentary sampling, may impose a barrier to elucidating the mechanisms underlying the phenomenon. For example, the sampling method adopted in all investigations assumes that ionic release follows a steady state pattern and that the concentration found at the specific time is representative of the release for the full term of treatment, a hypothesis that has not been verified. In addition, the expression of the results was

that of a metal mass per saliva volume, and thus no information about the most important parameter, that is time, can be incorporated in this model. Inevitably this restricts the applicability of the information obtained to the specific time period that sampling occurred. It might be that release has an additive effect and the values found relate only to the release potential for a specific time frame of an unknown extent. Since continuous monitoring and assessment of metal levels in saliva is not currently available, several issues must be resolved before any definitive conclusion is drawn on the potential for metal release from orthodontic appliances *in vivo*.

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