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ABSTRACT

Objective: The aim of our study was to assess the level of cytotoxicity of orthodontic appliances by checking the hypothesis that they induce an oxidative stress in mucosal cells.

Methods: Our study included two groups: 29 controls and 34 patients undergoing orthodontic treatment with fixed appliances. Samples were collected before bonding (T0), after one month (T1), and after three months (T2) of treatment.

Results: Results indicate the presence of oxidative stress following bonding, with significant differences in catalase activity ($p = 0.039$ at T1; $p = 0.01$ at T2) and in SOD activity ($p = 0.001$ at T1 and T2). The highest levels of enzymatic activities were recorded at T1 for both enzymes but subsided at T2, suggesting cellular self-repair capabilities in response to orthodontic alloys.

Conclusion: In view of our results, an appropriate choice of orthodontic alloys is required. Moreover, an identification of the subjects at risk for developing corrosion and galvanism and more attention are required to avoid ionic release in the oral cavity.

Keywords: cytotoxicity, orthodontic treatment, oxydative stress, catalase, SOD, fixed appliances.

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I. INTRODUCTION

The fixed orthodontic treatment is the therapeutic modality with the most clinical experience. It is the preferred technique of an entire era of classical orthodontics allowing practitioners to resolve aesthetic and functional complications associated with various malocclusions. (1)

In addition, a variety of biomaterials are used in the manufacture of the various components of the fixed orthodontic treatment including appliances, arches, tubes, metal ligatures, miniscrews, among other aids. Depending on clinical needs, the most dominant of these alloys are stainless steel and titanium nickel, with a chemical composition often comprising 68 to 80% of nickel, 12 to 26% chromium, and some other metals between 0, 1 and 14% (2).

Besides, the mechanical and physical properties of these metals are influenced by many different factors such as: temperature, salivary pH, ionic composition of saliva, microbiological and enzymatic activity, physical and chemical properties of food, conditions of oral health (3). This provides an environment conducive to corrosion phenomena and the release of metal ions in the mouth (4).

However, the placement of these metal alloys in the oral environment would have repercussions at

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the local and systemic level. Indeed, a large number of articles were focused on biological effects of orthodontic alloys currently used. (5,6,7)

A divergence is obviously noticed in many in vivo and in vitro studies investing this topic. Some author said that fixed appliances are inert and biocompatible (8,9) while others showed that appliances can caused cytotoxic and genotoxic effects in oral cells (10).

The main objective of this study was to test the hypothesis that fixed metal orthodontic appliances induce oxidative stress in the cells of the oral mucosa, causing activation of primary cellular defenses against oxidative stress.

II. MATERIALS AND METHODS

2.1 Study Design

To assess the cellular effect of metallic orthodontic devices, we conducted a longitudinal comparative clinical study on two groups over three months, with samples collected before (T0), after one month (T1), and after three months (T2) of treatment. This pilot study was approved by an institutional ethics committee (IORG0009738 N°68).

2.2 Study Population

Sixty-three subjects participated in the study, including 34 patients requiring fixed orthodontic treatment and 29 controls. The aim of the study was clearly explained for each patient, and so was the method of cell collection. Written consents were signed by each participant, and the treatment began after the approbation of the protocol by the ethical committee.

The average age was 21 years and 9 months (+- 4,1 years) in the experimental group (EG), and 24 years and 7 months (+- 3,9 years) in the control group (CG).

The eligibility criteria for subject selection involved; no systemic diseases, no prescribed medications, no oral diseases, nonsmokers, good oral hygiene, no prosthetics or oral metallic restorations, no oral piercing, no known allergies to nickel, or excessive exposure to metals.

First of all, the participants were selected with the use of a questionnaire to check whether or not they suit the included criteria. Then an oral examination was executed for each one.

The subjects of the treatment group were treated with fixed metallic appliances in both arches.

2.3 Samples Collection

To ensure the quality of the cell samples, participants were asked to energetically rinse their mouths with distilled water for 1minute, in order to remove all exfoliated cells. They were also instructed to avoid alimentation, and toothpastes or mouthwash containing fluoride, for two hours before cells collection (11). The buccal cells were harvested according to the method of Besarti et al (12), with gentle scarping of the internal part of both the right and left cheeks. Five strokes on each side were enough to get adequate cell density. This method uses interdental brushes intended to prevent a heterogeneous cell sample or any cell damage connected to the mechanical effect of scarping.

Buccal mucosa cells were collected before treatment (T0), 1 month (T1) and 3 months (T2) after bonding. Samples were placed in 2ml tubes (Eppendorf Hamburg, Germany) prefilled with 1,5 ml of phosphate-buffered saline solution, and immediately transported to the laboratory.

2.4 Laboratory Analyses

Each sample was evaluated for the state of oxidative stress by measuring antioxidant enzymatic activity. Two enzymes were targeted, forming the first barrier line of cell defense: superoxide dismutase (SOD), whose activity is measured in the visible region of the spectrum using glass tank and catalase, which is measured for the activity in the ultraviolet spectrum requiring the use of quartz tank.

2.5 Protein Extration

Cells were collected in a lysis buffer (Hepes 0.5 M containing 0.5 % Nonidet-P40, 1 mM PMSF, 1 µg/ml aprotinin, 2 µg/ml leupeptin, pH 7.4), and incubated for 20 min in ice before centrifugation.

Protein concentrations were determined in cell lysates using Protein Bio-Rad assay (13).

2.6 Measurement of SOD Activity

Superoxide dismutase (SOD) activity was determined according to the method described by Marklund and Marklund (14) by assaying the autooxidation and illumination of pyrogallol at 440 nm for 3 min. One unit of SOD activity was calculated as the amount of protein that caused 50 % pyrogallol autooxidation inhibition. The SOD activity is expressed as units per milligram protein.

2.7 Measurement of CAT

Catalase (CAT) activity was measured according to the method described by Aebi (15) by assaying the hydrolysis of H2O2 and the resulting decrease in absorbance at 240 nm over a 3-min period at 25 °C. The activity of CAT was calculated using the molar extinction coefficient (0.04/mM/cm). The results were expressed as micromole per minute per milligram protein.

2.8 Statistical Analysis

Data are expressed as the mean±standard deviation (SD) of the means. The analysis parameters were tested for homogeneity of variance and normality, and they were found to be normally distributed. Alteration at T0, T1, T2 of enzymatic activity in the treatment and control groups was evaluated using U-mann Whitney test; which is a non-parametric test used to compare the medians and the means of two samples.

The same test was used to compare enzymatic activity in the treatment group across time. Accordingly, a comparison between values at T0 and T1, between T0 and T2, and between T1 and T2 was established. In all cases, $p<0.05$ was considered statistically significant.

III. RESULTS

To further characterize the effect of orthodontics metallic appliances on the oxidative status, we measured changes in the activities of intracellular anti-oxidant enzymes: SOD and CAT.

The results showed a statistically significant difference (table 1) at enzymatic activity of catalase between the two groups at T1 ($p = 0.039$) and T2 ($p = 0.01$), and an insignificant one at T0 ($p = 0.828$); while being in favor of an increase in the enzymatic activity of catalases in the cell extracts of the group treated by a multi-metal vestibular treatment.

On the other hand, a statistically significant difference was revealed at the enzymatic activity of SOD between the CG and the EG at one month and at 3 months of treatment (with p values = 0.001 for T1 and T2 showed at table 1).

Table 1: Inter-Group Comparaison of Anti-Oxidant Enzyme Activity between Control and Experimental Group at t0, T1 and T2

		P Value	
	Groupe	SOD	Catalase
T0	CG	0,249	0,828
	EG		
T1	CG	0,001*	0,039*
	EG		
T2	CG	0,001*	0,001*
	EG		

** = significant p-value*

However, it was not significant at T0 (before treatment with a value of $p = 0.249$). The difference recorded is in favor of an increase in the enzymatic activity of superoxide dismutase in the group treated orthodontically.

In order to determine the fluctuation of catalase activity over time in the experimental group, statistical tests showed a significant difference between the catalase activities recorded before and after the treatment at one month and at three

months (p <0.001 respectively). Moreover, there was a statistically significan t variance when comparing SOD activities in pairs at T0, T1, and T2 (table 2).

Table 2: Changes in the Experimental Group of Anti-Oxidant Activity Between t0, T1 and T2

	SOD	Catalase
	p value	
T0 Vs T1	0,001*	<0,001*
T0 Vs T2	0,001*	<0,001*
T2 Vs T1	0,001*	0,021

** = significant p-value*

With the aim of understanding and analyzing this variation, a descriptive statistical study of the different split groups was made; where we distinguish 3 subgroups: EG at T0, EG at T1, and EG at T2. It showed a considerable increase in catalase activity observed one month after treatment, followed by a decrease in the antioxidant enzyme activity 3 months after bonding the orthodontic appliances.

These observations were also similar for SOD activity. (figures 1 and 2)

IV. DISCUSSION

The in vitro studies trying to simulate the intraoral biological conditions and the corrosive effects of this electrolytic medium seem insufficient, hence our choice of this in vivo study. However, in vivo studies do not lack risks or flaws, because the biological variations introduced by each patient affect the standardization of the study. Nevertheless, this appeared to be advantageous as it enabled fixed orthodontic appliances to be evaluated in their natural and functional environment. In fact, to eliminate interindividual variation, patients were assessed longitudinally to act as their own controls, so these variables were negligible during the overall assessment.

As previously announced, we were interested in the activities of anti-oxidant enzymes (SOD and catalase). According to the results of our study we can conclude that a state of oxidative stress was well recorded after one month of treatment. However, this state subsided after three months when the activities of the antioxidant enzymes registered a considerable decrease.

Most of the articles presenting in vivo studies such as ours have made use of viability tests (MTT test, the trypan blue exclusion dye test) (16), spectrophotometric determination of intracellular metal ions content (17) and genotoxic tests (comets assay and MN test) (18,19).

Tomakidi et al conducted an in vitro study (20) carried out on immortalized human gingival cells. It was concerned with the activity of an enzyme involved in the metabolism of glucose on the level of the cell lysosome: the hexosaminidase. It is therefore possible to infer the level of cell viability and estimate the acute cytotoxic effects after exposure to a test compound, while monitoring the variation in the activity of this enzyme. The results showed that the latter exhibits significant activity in the presence of metallic orthodontic material.

A recent study carried out in vivo (21) involved 60 subjects, 40 of them were treated with orthodontic fixed appliances and 20 controls, with three sampling times: before treatment, 3 months and 6 months after the beginning of treatment.

A cell viability test using Trypan Blue exclusion dye test was performed. It showed that the level of cell viability decreases over time, although the effects at the DNA level (comet test) are more significant after 3 months of treatment to subside at 6 months; which is similar to our study observations of oxidative effects. This variation can be related to reparative mechanisms activated to maintain DNA integrity.

One of the first investigators in biological effects of orthodontics appliances were Faccioni et al (22). During this in vivo study, viability tests,

spectrophotometric determination of intracellular content of nickel and cobalt and comet assay were used. They showed that nickel and cobalt released from fixed orthodontic appliances can induce DNA damage in oral mucosa cells. It also prompts a decrease of cellular viability.

Indeed, determination of the ionic intra-cellular content seems an important step to confirm and expand these findings of stress oxidative state.

Buczko et al (23) studied the effect of orthodontic appliances. They measured the salivary nickel concentration and evaluated the expression of caspase-3 (indicator of apoptosis) immuno-histochemically. Samples were taken at three different times: before treatment, after one week, and after 24 weeks of treatment. A variation of biological effects was similar to that which we obtained during our study. Researchers recorded an increase in damage at the start of treatment which subsides afterwards. This variation has been attributed to self-repair phenomena but also to the levels of nickel released (which increase after one week, then decrease after 24 weeks of treatment) and to mechanical attacks of the appliances and arches. These last were in direct contact with oral mucosa.

As a matter of fact, a vivo-long term studies are recommended in order to monitoring chronic exposure over several years of orthodontic treatment (surgical orthodontic cases).

For the last few decades and with the multiplication of risks and sources of danger, we have attributed great interest to biology and human health (24). In this same perspective, our clinical investigations aim to determine the effect of the material introduced in the mouth for functional and aesthetic orthodontic purposes for an average duration of 24 months. A set of recommendations will be useful for clinical practice.

Therefore, the chemical composition of orthodontic alloys which require further consideration by orthodontist (25) and shorten the duration of treatment by using more appropriate orthodontic mechanics, is recommended.

Moreover, an identification of patients at risk of developing allergic reactions, corrosion and galvanism, and more attention are required to avoid ionic release in the oral cavity.

V. CONCLUSION

Referring to this work, an oxidative stress was well recorded after the bonding of the orthodontics metallic appliances.

This state of oxidative stress is variable. It has showed a significant increase at first, then a decrease after 3 months of treatment

Thus, this study highlights a several intra-cellular effects of orthodontic appliances. However further investigation with largest study population and extended follow-ups may reveals more data evidences.

Abbreviations

EG: experimental group
CG: control group
SOD: Superoxide dismutase

Declaration of Interests and Conflicts

The authors declare that there are no personal, institutional, business-oriented, or financial conflicts of interest influencing the results of this study. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

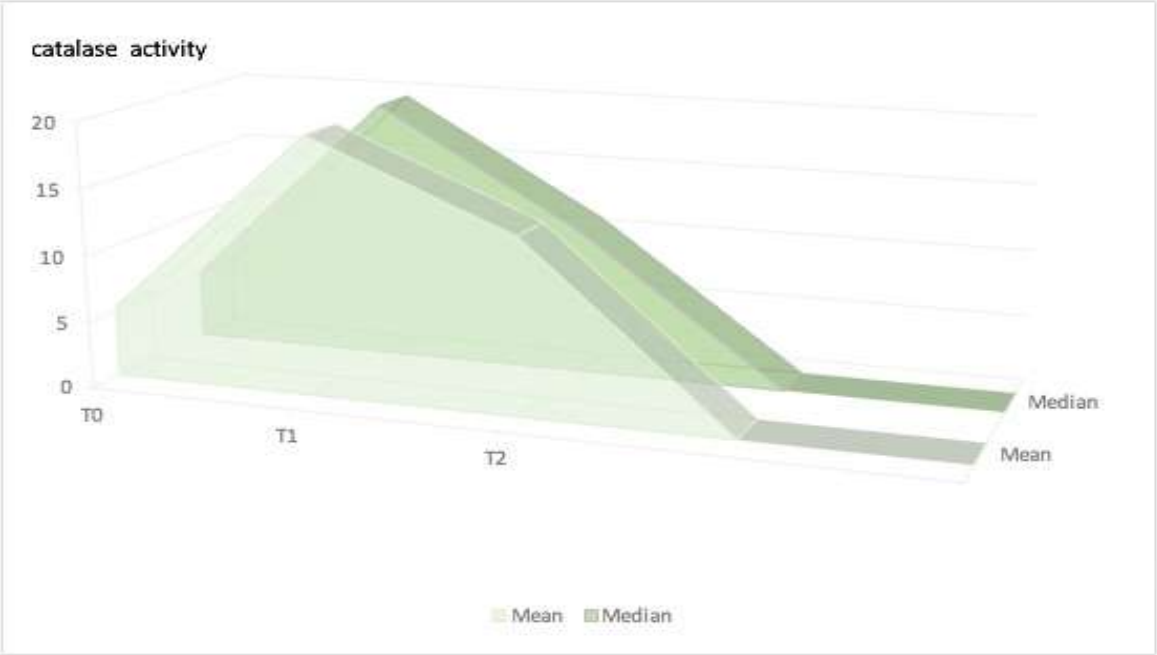

Figure 1: Catalase Activity Evolution Depending on Treatment Duration

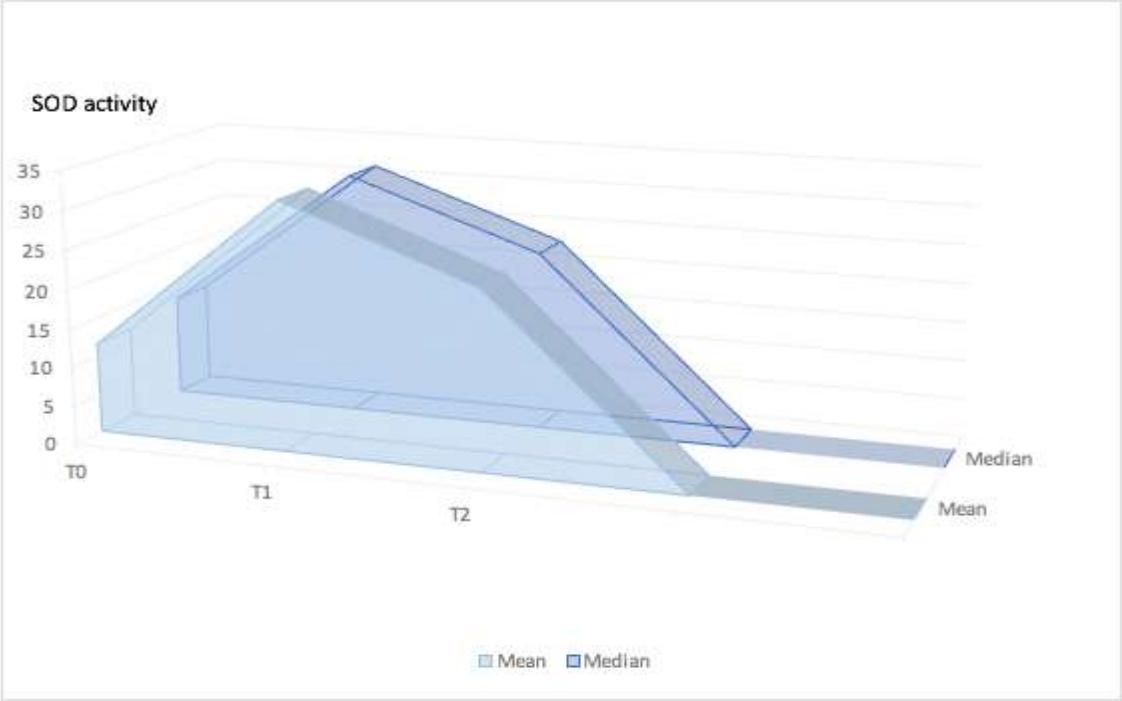

Figure 2: SOD Activity Evolution Depending on Treatment Duration

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