Camila Alessandra Pazzini

# CONDIÇÕES SANGUÍNEAS E GENGIVAIS EM PACIENTES ALÉRGICOS AO NÍQUEL SUBMETIDOS A TRATAMENTO ORTODÔNTICO COM BRAQUETES CONVENCIONAIS E NÍQUEL FREE

BELO HORIZONTE

2012

Camila Alessandra Pazzini

# CONDIÇÕES SANGUÍNEAS E GENGIVAIS EM PACIENTES ALÉRGICOS AO NÍQUEL SUBMETIDOS A TRATAMENTO ORTODÔNTICO COM BRAQUETES CONVENCIONAIS E NÍQUEL FREE

Tese apresentada ao Programa de Pós-Graduação em Odontologia - Área de concentração em Odontopediatria da Faculdade de Odontologia da Universidade Federal de Minas Gerais como requisito parcial à obtenção do título de Doutor em Odontologia.

Orientador: Prof. Dr. Saul Martins de Paiva
Co-orientador: Prof. Dr. Leandro Silva Marques

Faculdade de Odontologia
Universidade Federal de Minas Gerais
Belo Horizonte
2012

**Ficha Catalográfica**

**Folha de aprovação**

# Ata de Aprovação

*Dedico este trabalho*
*aos meus pais, Gilberto e Elba, meus irmãos, Danielle e Rodrigo, meu noivo Dawi e minha querida avó Maria de Nazareth,*
*que participaram comigo dessa importante conquista, dando-me incentivo para seguir em frente.*

# AGRADECIMENTOS

A Deus, por estar sempre presente em minha vida, iluminando o meu caminho. Responsável por tudo que estou vivendo até hoje!

Ao meu noivo Dawi pela presença constante, paciência e amor!

Aos meus pais, Elba e Gilberto que nunca mediram esforços para a realização dos meus sonhos e por acreditarem em mim mais uma vez, muitas vezes esquecendo suas próprias vidas.

Aos meus irmãos Danielle e Rodrigo, que mesmo distantes, são o meu porto seguro! Obrigada e eu amo vocês!

Aos meus orientadores Professor Saul Martins de Paiva e Leandro Silva Marques pelo empenho, dedicação, compreensão e por me darem a grande oportunidade de crescimento pessoal e profissional junto de vocês. Sinto-me privilegiada de poder ter vocês por perto nesta jornada da vida.

Professor Leandro Silva Marques.... não sei como posso te agradecer por tudo e por me fazer uma pessoa melhor. Obrigada pela instrução, pelo incentivo e por sempre acreditar em mim fazendo-me sempre perceber o melhor caminho e sentido dos momentos e oportunidades! O mesmo agradecimento repasso a Maria Letícia Ramos-Jorge! Obrigada por ser um exemplo pra mim! Minha eterna gratidão!

Ao meu grande incentivador e mestre Professor Gilberto de Oliveira Júnior, que desde a minha formação, trabalhou me incentivando e acreditando nos meus sonhos, me dando força e incentivo para a conclusão de mais esta conquista! Nunca saberei como te agradecer! O meu carinho e admiração pelo profissionalismo e humildade que me fez seguir os caminhos da ortodontia.

Professor Luciano José Pereira, pela disponibilidade e por estar trabalhando ativamente no nosso trabalho. Muito obrigada pela oportunidade de conviver com você! Obrigada por tudo!

Aos Professores do Departamento de Odontopediatria e Ortodontia pelo incentivo e apoio. Especialmente às Professoras Isabela Almeida Pordeus, Carolina de Castro Martins, Miriam Pimenta Parreira do Vale, Patrícia Maria Pereira de Araujo Zarzar, Júnia Maria Cheib Serra Negra e Sheyla Márcia Auad que estiveram presentes contribuindo em momentos importantes durante essa trajetória.
A professora Tarcília Aparecida da Silva, pelo interesse pelo nosso trabalho e pela imensa ajuda!

As funcionárias da Faculdade de Odontologia da Universidade Federal de Minas Gerais Beth, Zuleica, Laís sempre pacientes e dispostas a ajudar.

Aos pacientes que participaram da pesquisa bem como seus pais e responsáveis. O meu muito obrigada!

As amigas do doutorado em especial à Cláudia Marina de Sousa Viegas, Cristiane Bacin Bendo e Fernanda Sardenberg de Matos, pela disponibilidade e companheirismo. Com certeza essa trajetória não seria a mesma sem vocês. Obrigada pelos momentos enriquecedores!

A amiga Elizandra pela grande convivência, amizade e incentivo!

A todos os meus familiares e amigos que souberam entender a minha ausência e torceram por esta minha conquista.

**Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo apoio financeiro.**

*"A diferença entre o possível e o impossível está na vontade humana."*

# ARTIGO 1

# Gingival status and blood parameters in orthodontic patients with and without nickel allergy following braces removal

Short title**:** Nickel effects after braces removal

*Camila Alessandra Pazzini*[a], *Luciano José Pereira*[b], *Ana Paula Peconick*[c], *Leandro Silva Marques*[d], *Saul Martins Paiva*[a]

[a] Department of Orthodontics and Pediatric Dentistry, Federal University of Minas Gerais – UFMG, Belo Horizonte, MG, Brazil.

[b] Department of Physiology and Pharmacology, Federal University of Lavras – UFLA, Lavras, MG, Brazil.

[c] Department of Immunology, Federal University of Lavras – UFLA, Lavras, MG, Brazil.

[d] Department of Orthodontics, Federal University of Vale do Jequitinhonha e Mucuri – UFVJM, Diamantina, MG, Brazil.

The authors report no commercial, proprietary or financial interest in the products or companies described in this article.

**Corresponding Author:** Camila Alessandra Pazzini
Department of Orthodontics and Pediatric Dentistry, Federal University of Minas Gerais, UFMG, Belo Horizonte, MG, Brazil.
Rua Benjamin Constant, 33, centro – CEP 37200000, Lavras, MG, Brazil
Phone: +55 35 98042133
E-mail: camilapazzini@hotmail.com

**# Artigo formatado segundo as normas de publicação do periódico: Brazilian Dental Journal (Anexo C)**

# Gingival status and blood parameters in orthodontic patients with nickel allergy following braces removal


## ABSTRACT

The aim of the present study was to assess gingival status and blood parameters one month after the removal of braces in patients with nickel allergy. Ninety-six patients were initially evaluated. Allergy to nickel was diagnosed using a patch test. After the determination of the prevalence of those allergic to nickel, two groups were formed: 16 allergic and 16 non-allergic patients. Gingival status was determined using the gingival index. Humoral characteristics were determined through a complete blood test, including the quantification of nickel in the blood and IgE levels. Assessments of periodontal status were determined by a single, blinded, duly calibrated examiner and blood samples were taken after nine months of treatment and one month after removing the orthodontic appliances. Statistical analysis involved paired and non-paired t-tests, the Mann-Whitney, Wilcoxon, McNemar and linear trend chi-square tests ($p \leq 0.05$). The number of eosinophils, basophils and monocytes increased, whereas the number of bands decreased between periods in the allergic group compared with controls ($p < 0.05$). The number of lymphocytes increased significantly in the control group ($p < 0.05$). Gingival index scores decreased significantly in both groups during and after treatment. The results of the present study analyzing the period of direct contact with the allergenic agent and after its removal indicate that orthodontic treatment with conventional stainless steel appliances does not initiate or aggravate a hypersensitive reaction to nickel.


**Keywords:** nickel, allergy, blood, orthodontics

## INTRODUCTION

Industrialization and modern life have resulted in an increase in dermal exposure to metals and a consequent increase in the incidence of allergies, especially to nickel

(1), which can cause allergic contact dermatitis (2,3). Contact dermatitis is generally produced by external exposure of the skin to an allergen. However, an administered allergen can reach the skin through the systemic circulation, producing systemic contact dermatitis (4). The prevalence of nickel allergy in the general population ranges from 8% to 17% in females and 1% to 5% in males (5).

Chemicals of small molecular weight (i.e., haptens) can irritate tissues by inducing the production of various pro-inflammatory and chemotactic molecules and are potentially allergenic when able to bind to proteins, such as immune response molecules (6,7). Nickel can induce T lymphocytes to produce cytokines, such as INF-γ, IL-2, IL-5 and IL-10, thereby stimulating tissue proliferation, which may favor gingival hyperplasia. It would therefore be plausible to suppose that the continuous release of small amounts of nickel into the epithelium could constitute an initiating factor of gingival over-growth induced by orthodontic braces (8).

A recent study has indicated nickel to be an element that is potentially capable of affecting periodontal status and blood cells in allergic patients during orthodontic treatment (9). Ninety-six randomly selected patients participated in the study. After determining the prevalence of those allergic to nickel, 2 groups were formed: 16 allergic patients and 16 nonallergic patients. Allergies to nickel were diagnosed by using the patch test, periodontal conditions were determined by using the gingival index, and humoral characteristics were determined through a complete blood test, including the quantification of nickel in the blood and the immunoglobulin E level. Statistically significant differences between groups were found for bands. There was no correlation between the concentration of nickel and immunoglobulin E level. However, there was a positive correlation between the gingival index and the number of bands.

However, the results seem to be more related to a local inflammatory response rather than a systemic allergic reaction. Moreover, it remains unknown whether the cumulative effect of nickel during orthodontic treatment is reversed after the braces are removed or whether there are significant lasting effects on periodontal status.

Thus, the aim of the present study was to evaluate periodontal status and blood parameters one month after the removal of braces in patients with nickel allergy.

## MATERIALS AND METHODS

This study received approval from the Human Research Ethics Committee of the *Centro Universitário de Lavras* (Brazil) under process number 0001.0.380.000-07. The legal guardians and the adolescents signed informed consent forms authorizing participation in the study.

A prospective cohort study was carried out. The characteristics of the sample and the methodological criteria are described in a previous publication (9). Figure 1 displays the flowchart illustrating the study design and sequence of procedures.

Ninety-six randomly selected patients were initially evaluated. Six patients refused to participate in the study. All were white; 58 (60%) were female and 32 (40%) were male; their ages ranged from 10 to 43 years. Allergy to nickel was diagnosed using a patch test. Sixteen patients were allergic to nickel and thus were randomly selected from 16 non-allergic patients, formed two groups. For the purposes of comparison between the treatment period and after the removal of the braces, all 32 participants underwent full blood tests nine months after beginning treatment (9) and one month after the removal of the braces for the assessment of the leukogram and total immunoglobulin E (IgE) and the determination of the amount of circulating nickel in the blood.

Assessments of periodontal status were carried out by a single, blinded, duly calibrated (Kappa > 0.90) examiner at regular three-month intervals for a period of 12 months (total of four evaluations) during treatment, as described elsewhere (9). Since each patient finished treatment at different moments, the final evaluation was standardized as one month following the completion of treatment. Prophylaxis with a bicarbonate spray was performed in each session (following the gingival evaluation). All patients were monitored monthly for biofilm (plaque) control and hygiene guidance. Clinical gingival characteristics (color and volume) were assessed using the Löe gingival index, which takes into account qualitative changes in the gingival tissue (10).

Statistical analysis involved t-tests, paired t-tests, Mann-Whitney and Wilcoxon tests for the intergroup/intragroup comparisons of blood components after nine months

of treatment and one month after the removal of the orthodontic appliances. The linear trend chi-square test was used to compare periodontal status between groups in the same periods. The McNemar test was used for the comparison of the gingival index (dichotomized as absence/presence) within each group between the two evaluation times. Differences were considered significant when $p \leq 0.05$.

## RESULTS

No differences were found regarding blood parameters between groups during or after orthodontic treatment ($p>0.05$) (Table 1). However, the number of eosinophils, basophils and monocytes increased significantly between nine months of treatment and one month after the removal of the orthodontic appliances in the allergic group ($p<0.05$), whereas the number of bands decreased significantly ($p<0.05$). The number of lymphocytes increased significantly in the control group ($p<0.05$) and the number of segmented neutrophils decreased ($p<0.05$). Plasma nickel levels were increased one month after removing orthodontic appliances in both groups ($p<0.05$) (Table 1). Gingival index scores decreased significantly one month after removing the braces in both groups ($p<0.05$) (Table 2).

## DISCUSSION

In a previous study by our group using the same sample, patients with an allergy to nickel had higher gingival index scores and band counts during orthodontic treatment than non-allergic patients (9). However, the current data indicate a significant reduction in both parameters after the removal of orthodontic appliances, demonstrating that periodontal and blood alterations tend to be eliminated after the removal of the braces. As orthodontic appliances hamper oral hygiene, dental biofilm accumulates with greater facility on tooth surfaces and appliances in most patients and the consequent periodontal disease leads to an increase in neutrophils (11). Once the appliance is removed, however, the conditions favoring the formation of biofilm are no longer present and there is a consequent reduction in gingival index scores and the amount of bands. The reduction in the number of bands may be explained by the fact that these cells are present in bacterial infection and/or acute inflammation (12). Moreover, the use of nickel alloy for longer periods tends to induce oral tolerance through mechanisms that

modulate sensitivity (13). The findings of the present study corroborate these data, as there was a significant reduction in the gingival index on the final evaluation in both groups.

A decrease in IgE levels occurred between the evaluations performed during treatment and after the removal of the orthodontic appliances in both groups, with a statistically significant difference in the control group. Moreover, an increase in circulating levels of nickel was found between the two evaluations in both groups. It seems that hypersensitive reactions to nickel over a long period of time are only likely to occur with prior sensitization from non-dental contact, which is rare, and small doses of nickel may induce tolerance to this allergen (14). With orthodontic braces, continuous exposure within the oral cavity may be a pivotal factor regarding tolerance in individuals who have not been previously sensitized (15). Nickel can be lost through sweat and saliva, but is concentrated in the blood as a result of the acute effects of exposure (16). Therefore, one cannot discard the possibility of accumulation in the body. A number of authors believe that a longer contact period may induce tolerance to this metal. However, the amount needed to induce tolerance in human tissues is not yet known (17).

Analyzing the white blood cells in allergic and non-allergic patients during and after treatment (Table 1), an increase of eosinophils, monocytes and basophils was observed. This is believed not to be due to the removal of the appliances itself, but rather to the long exposure to nickel during treatment, as there was an increase in plasma nickel concentration in both groups. The continuous low-level stimulus of antigens, such as nickel, raises the level of IL-4 produced by T cells, regardless of whether or not an individual is allergic, which favors a polarized immune response for a $T_H2$ profile (18). In the present study, increases in lymphocytes, eosinophils and IgE occurred in both the allergic and non-allergic patients, may suggest the installation of a $T_H2$ immune response. These results corroborate findings described by Niiyama et al., who also assessed the systemic response to nickel (19). A number of studies also report a nickel-produced response with a predominance of $T_H1$ $CD4^+$ T cells due to the presence of interferon-γ, but the balance generally tends to favor the expression of $T_H2$ cells and inhibit other subpopulations (13-20,21).

Analyses of cytokine production by Ni-specific T cells have demonstrated a mixed $T_H1$ and $T_H2$ cytokine profile in both T-cell clones and peripheral blood mononuclear cells (22). However, the allergic patients exhibited a greater response than the non-allergic patients due to the fact that they were more sensitive to the allergen (nickel), as only the allergic group exhibited a significant increase in eosinophils, monocytes and basophils (Table 1). A similar result occurred regarding the number of lymphocytes, which also increased between evaluation times. This may be explained by the fact that individuals allergic to nickel have a small number or lack of specific suppressor T cells, which regulate the number of leukocyte populations (23).

The results of the present study analyzing the period of direct contact with the allergenic agent and after its removal indicate that orthodontic treatment with conventional stainless steel appliances does not initiate or aggravate a hypersensitive reaction to nickel. However, as periodontal alterations may be associated to nickel, it is important for orthodontists to seek alternatives for the treatment of patients who exhibit compromised periodontal health. There are a number of non-allergic braces for nickel-sensitive patients, such as titanium braces, which are more corrosion resistant and do not release nickel into the oral cavity. Twenty-two zero five (2205) duplex stainless steel contains much less nickel and could also be used for orthodontic appliances with a lesser allergic potential. Other options include ceramic or plastic braces and those with a low nickel concentration, known as nickel-free braces (24). However, such braces offer poorer mechanics in comparison to conventional braces.

No differences in blood or gingival parameters were found between orthodontic patients allergic to nickel and non-allergic patients one month after removing the braces.

RESUMO

O objetivo do estudo foi avaliar a condição periodontal e os parâmetros sanguíneos de um mês após a remoção do aparelho ortodôntico naqueles pacientes com e sem alergia ao níquel. Noventa e seis pacientes participaram do estudo. Depois de determinar a prevalência de pessoas alérgicas ao níquel, formaram-se dois grupos: 16 alérgicos e 16 pacientes não alérgicos. Alergia ao níquel foi diagnosticada através de um teste de contato. A condição gengival foi determinada utilizando o Índice Gengival.

Características humorais foram determinadas através de um hemograma completo, incluindo a quantificação de níquel nos níveis sanguíneos e IgE. Avaliações da condição gengival (realizados por um único examinador de maneira cega e devidamente calibrado (Kappa> 0,90) e amostras de sangue foram colhidas durante o tratamento ortodôntico (9 meses) e um mês após a remoção de aparelhos ortodônticos. A análise estatística envolveu testes t pareado e não pareado, Mann-Whitney, Wilcoxon, McNemar e qui-quadrado de tendência linear ($p \leq 0,05$). O número de eosinófilos, basófilos e monócitos aumentou enquanto o número de bastões diminuiu em ambos os períodos no grupo alérgico ($p<0,05$). O número de linfócitos aumentou significativamente no grupo controle ($p<0,05$). O índice gengival diminuiu significativamente em ambos os grupos durante e após o tratamento. Os resultados do presente estudo analisando o período de contato direto com o agente alergênico e após a sua remoção indicam que o tratamento ortodôntico com aparelhos de aço inoxidável convencionais não iniciar ou agravar uma reação de hipersensibilidade ao níquel.

## ACKNOWLEDGMENTS

This study was supported by the following Brazilian fostering agencies: National Council for Scientific and Technological Development (CNPq), Ministry of Science and Technology, State of Minas Gerais Research Foundation (FAPEMIG) and Coordination for Improvement of Higher Education Personnel (CAPES).

## REFERENCES

1- Uter W, Pfahlberg A, Gefeller O, Geier J, Schnuch A. Risk factors for contact allergy to nickel - results of a multifactorial analysis. Contact Dermatitis 2003;1:33-8.

2- Thyssen JP, Linneberg A, Menné T, Johansen JD. The epidemiology of contact allergy in the general population--prevalence and main findings. Contact Dermatitis 2007;57:287-99.

3- Jacob SE, Zapolanski T. Systemic contact dermatitis. Dermatitis 2008;19:9-15.

4- Nijhawan RI, Molenda M, Zirwas MJ, Jacob SE. Systemic contact dermatitis. Dermatol Clin 2009;27:355-64.

5- Thyssen JP, Johansen JD, Carlsen BC, Menné T. Prevalence of nickel and cobalt allergy among female patients with dermatitis before and after Danish government regulation: a 23-year retrospective study. J Am Acad Dermatol 2009;61:799-805.

6- Kapsenberg ML. Chemicals and proteins as allergens and adjuvants. Toxicology Lett 1996;86:79-83.

7- Menezes LM, Campos LC, Quintão CC, Bolognese AM. Hypersensitivity to metals in orthodontics. Am J Orthod Dentofacial Orthop 2004;126:58-64.

8- Gursoy UK, Sokucu O, Uitto VJ, Aydin A, Demirer S, Toker H, Erdem O, Sayal A. The role of nickel accumulation and epithelial cell proliferation in orthodontic treatment-induced gingival overgrowth. Eur J Orthod 2007;29:555-8.

9- Pazzini CA, Pereira LJ, Carlos RG, de Melo GE, Zampini MA, Marques LS. Nickel: periodontal status and blood parameters in allergic orthodontic patients. Am J Orthod Dentofacial Orthop 2011;139:55-9.

10- Löe H. The Gingival Index, the Plaque Index and the Retention Index Systems. J Periodontol 1967;38:610-6.

11- Ujiie Y, Oida S, Gomi K, Arai T, Fukae M. Neutrophil elastase is involved in the initial destruction of human periodontal ligament. J Periodontal Res 2007;42:325-30.

12- Ramadan AA. Effect of nickel and chromium on gingival tissues during orthodontic treatment: a longitudinal study. World J Orthod 2004;5:230-4.

13- Marigo M, Nouer DF, Genelhu MC, Malaquias LC, Pizziolo VR, Costa AS, Martins-Filho OA, Alves-Oliveira LF. Evaluation of immunologic profile in patients with nickel sensitivity due to use of fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 2003;124:46-52.

14- Setcos JC, Babaei-Mahani A, Silvio LD, Mjör IA, Wilson NH. The safety of nickel containing dental alloys. Dent Mater 2006;22:1163-8.

15- Van Hoogstraten IM, Andersen KE, Von Blomberg BM, Boden D, Bruynzeel DP, Burrows D, Camarasa JG, Dooms-Goossens A, Kraal G, Lahti A, et al.

Reduced frequency of nickel allergy upon oral nickel contact at an early age. Clin Exp Immunol 1991;85:441-5.

16- Eliades T, Athanasiou AE. In vivo aging of orthodontic alloys: implications for corrosion potential nickel release and biocompatibility. Angle Orthod 2002;72:222-37.

17- Kerosuo H, Moe G, Hensten-Pettersen A. Salivary nickel and chromium in subjects with different types of fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 1997;111:595-8.

18- Chakir H, Wang H, Lefebvre DE, Webb J, Scott FW. T-bet/GATA-3 ratio as a measure of the Th1/Th2 cytokine profile in mixed cell populations: predominant role of GATA-3. J Immunol Methods 2003;278:157-69.

19- Niiyama S, Tamauchi H, Amoh Y, Terashima M, Matsumura Y, Kanoh M, Habu S, Komotori J, Katsuoka K.Th2 immune response plays a critical role in the development of nickel-induced allergic contact dermatitis. Int Arch Allergy Immunol 2010;153:303-14.

20- Minang JT, Areström I, Zuber B, Jönsson G, Troye-Blomberg M, Ahlborg N. Nickel-induced IL- 10 down-regulates Th1- but not Th2 -type cytokine responses to the contact allergen nickel. Clin Exp Immunol 2006b;143:494-502.

21- Schwenk M, Sack U, Esser C, Klein R. Diagnostic relevance of the determination of lymphocyte subpopulations in environmental medicine. Int J Hyg Environ Health 2007;210:177-98.

22- Rustemeyer T, Von Blomberg BM, Van Hoogstraten IM, Bruynzeel DP, Scheper RJ. Analysis of effector and regulatory immune reactivity to nickel. Clin Exp Allergy 2004;34:1458-66.

23- Cavani A. Breaking tolerance to nickel. Toxicology 2005;209:119-21.

24- Pazzini CA, Marques LS, Pereira LJ, Corrêa-Faria P, Paiva SM. Allergic reactions and nickel-free braces: a systematic review. Braz Oral Res 2011;25:85-90.

Table 1: Comparative analysis of blood components in experimental (allergic) and control (non allergic) groups during and after orthodontic treatment

| | Experimental Group (AL) | | | Control Group (NAL) | | | | |
|---|---|---|---|---|---|---|---|---|
| | During mean±dp | After mean±dp | D-A p-value | During mean±dp | After mean±dp | D-A p-value | During (AL) x During (NAL) | After (AL) x After (NAL) |
| Leukocytes(mil/mm³) | 6411.75±1759.20 | 6052.90±1872.85 | 0.412[c] | 5657.14±1264.73 | 5862.50±1142.60 | 0.957[c] | 0.821[a] | 0.299[a] |
| Eosinophils(mil/mm³) | 132.70±60.38 | 259.35±187.83 | **0.046**[c] | 136.00±104.00 | 197.80±195.77 | 0.967[c] | 0.204[a] | 0.322[b] |
| Basophils(mil/mm³) | 6.25±17.95 | 33.50±32.00 | **0.001***[c] | 11.95±22.42 | 23.75±35.75 | 0.150[c] | 0.392[b] | 0.168[b] |
| Lymphocytes(mil/mm³) | 2115.58±633.35 | 2115.58±633.35 | 0.226[c] | 1874.37±461.98 | 2170.00±461.05 | **0.039***[c] | 0.264[a] | 0.345[a] |
| Segmenteds(mil/mm³) | 3702.47±1116.30 | 3066.95±1303.25 | 0.073[c] | 3400.25±1010.65 | 3254.75±947.85 | **0.030***[c] | 0.991[a] | 0.264[a] |
| Bands(mil/mm³) | 127.47±48.90 | 24.35±47.50 | **0.000***[c] | 67.45±48.27 | 53.20±80.80 | 0.240[c] | 0.982[a] | 0.277[b] |
| Monocytes(mil/mm³) | 327.30±87.20 | 442.95±111.10 | **0.002***[c] | 355.25±137.35 | 374.25±138.38 | 0.295[c] | 0.900[b] | 0.917[a] |
| IgE(UI/mL)[1] | 631.30±821.11 | 597.90±675.10 | 0.637[c] | 446.67±425.00 | 392.90±353.07 | **0.001***[c] | 0.061[a] | 0.078[a] |
| Nickel(mcg/L) | 1.68±3.4 | 3.81±1.05 | **0.010***[d] | 0.68±2.95 | 2.82±1.65 | **0.039***[d] | 0.212[b] | 0.282[b] |

[a] T test; [b] Mann Whitney test; [c] Paired t test; [d] Wilcoxon.

*($p < .05$); AL= allergic; NAL= non allergic.

All variables were tested with Kolmogorov-Smirnov normality.

[1] IgE was transformed by a square root.

Table 2: Comparative analysis of gingival index (GI) in the experimental (AL) and control groups (NAL) during orthodontic treatment and one month after removing orthodontic appliances

| EXAMS | Groups | Results GI | | | | AL x NAL[a] |
|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | P Value |
| During | Experimental [A] | 1 (5.9%) | 7(41.2%) | 7(41.2%) | 2(11.8%) | **0.026*** |
| | Control | 5(31.3%) | 7(43.8%) | 4(25%) | 0(0%) | |
| After | Experimental [B] | 8(47.1%) | 8(47.1%) | 1(5.9%) | 0(0%) | 0.160 |
| | Control | 11(68.8%) | 5(31.3%) | 0(0%) | 0(0%) | |

[a] Linear trend Chi-square Test comparing gingival index between groups during and after orthodontic treatment.

[A,B] McNemar test for gingival index dichotomized into absence (0) and presence of periodontal inflammation (1, 2 or 3) inside each group comparing both moments: during and after orthodontic treatment. (Experimental p=**0.016***; Control p=0.070).

*(p<.05); AL= allergic; NAL= non allergic.

Figure legend:

Fig: Flowchart of study

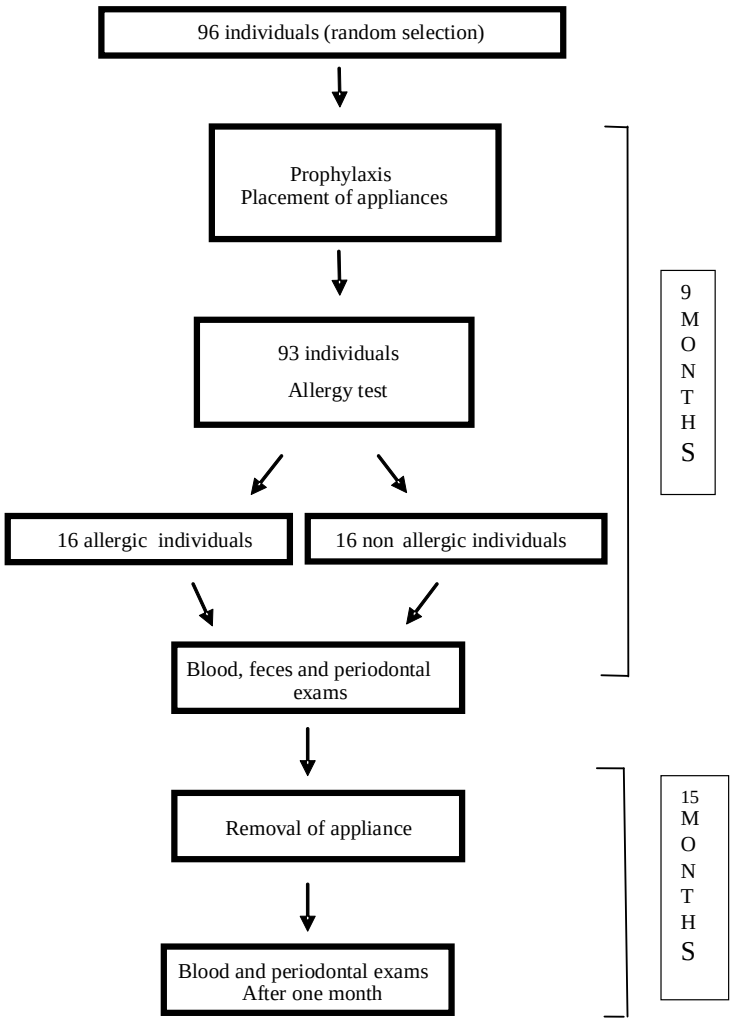



Figure 1: Flowchart of study

# ARTIGO 2

# GINGIVAL STATUS AND BLOOD PARAMETERS IN ORTHODONTICS PATIENTS WITH NICKEL ALLERGY TREATED WITH CONVENTIONAL AND NICKEL-FREE BRACES

Camila Alessandra Pazzini[a], Leandro Silva Marques[b], Maria Letícia Ramos-Jorge[c,] Luciano José Pereira[d], Saul Martins Paiva[a]

[a] Department of Orthodontics and Pediatric Dentistry, Federal University of Minas Gerais – UFMG, Belo Horizonte, MG, Brazil.

[b] Department of Orthodontics, Federal University of Vale do Jequitinhonha e Mucuri – UFVJM, Diamantina, MG, Brazil.

[c] Departmento of Pediatric Dentistry, Federal University of Vale do Jequitinhonha e Mucuri – UFVJM, Diamantina, MG, Brazil.

[d] Department of Physiology and Pharmacology, Federal University of Lavras – UFLA, Lavras, MG, Brazil.

The authors report no commercial, proprietary or financial interest in the products or companies described in this article.

**Corresponding Author:**
Prof. Dr. Saul Martins Paiva
Avenida Bandeirantes, 2275/500 – Mangabeiras CEP 30210420, Belo Horizonte, MG, Brazil Phone: +55 31 99673382
E-mail: smpaiva@uol.com.br

**# Artigo formatado segundo as normas de publicação do periódico: American Journal of Orthodontics and Dentofacial Orthopedics (Anexo D)**

ABSTRACT

**Introduction:** Allergic and inflammatory reactions have commonly been associated with release of metal ions, especially nickel, during orthodontic treatment. **Objective:** To compare the gingival and blood status in allergic individuals treated with conventional and nickel-free braces. **Materials and Methods:** Eighty patients were initially evaluated. Forty-two individuals allergic to nickel were randomly divided into two groups: conventional braces (n=21) and nickel-free braces (n=21). Gingival status (gingival hyperplasia, change in color and bleeding) was assessed before treatment ($T_0$) and at 3-month intervals for 12 months ($T_1$, $T_2$, $T_3$, and $T_4$), using the Löe Index. Humoral characteristics were determined through a complete blood test, including the quantification of nickel and the immunoglobulin E level. Data were analyzed using the Mann-Whitney test, paired and unpaired t test, Wilcoxon, Pearson and Spearman correlation ($p \leq .05$). **Results:** There were no significant differences in blood inter-assessment groups before treatment ($T_0$, $p > .05$). During orthodontic treatment ($T_3$), the segmented cells presented statistically significant decrease in nickel-free group (0.040). There was an increased number of basophils and the concentration of nickel from $T_0$ to $T_3$ ($p \leq .05$) in both groups. However, the number of eosinophils and IgE levels decreased significantly ($p \leq .05$). There was no significant correlation between the concentration of nickel and gingival index, level of IgE, basophils, eosinophils, bands and segmenteds ($p > .05$). **Conclusions:** Individuals treated with nickel-free braces had lower blood disorders and gingival status than individuals treated with conventional braces, but still within the normal range.

**Key Words**: Nickel allergy, orthodontic treatment, immunology, nickel-free braces

## INTRODUCTION

Chemicals of small molecular weight may irritate tissues by inducing the production of various pro-inflammatory and chemotactic molecules and are potentially allergenic when able to bind to proteins, such as immune response molecules.[1-4] Nickel can induce T lymphocytes to produce cytokines, including INF-γ as well as IL-2, IL-5, and IL-10, thereby stimulating tissue proliferation, which may favor gingival hyperplasia and blood disorders. It would therefore be plausible to suppose that the continuous release of small amounts of nickel into the epithelium could constitute an initiating factor of gingival over-growth induced by orthodontic braces.[5]

Recent studies have pointed nickel to cause changes in periodontal and immunologic conditions of patients allergics.[6-12] An immune response induced by nickel appliances is generally called contact dermatitis and, from the immunologic standpoint, is considered type IV hypersensitivity.[13]

At the same time, there has been a tendency of the industry to produce orthodontic materials with low concentrations of nickel (0.2% to 4%). Such appliances are denominated nickel-free and are advertised as releasing low quantities of nickel ions, which may diminish the allergic response in sensitive patients.[14-16,10] In this studies involving nickel-free braces may provide important information by first determining whether nickel is truly the agent responsible for triggering responses of an inflammatory and/or allergic nature. Moreover, such studies would determine whether nickelfree braces actually represent a viable alternative for patients who are allergic to nickel.

The objectives of this study were to compare and correlate along the gingival and blood condition of nickel-allergic patients treated with conventional and nickel-free braces before e during orthodontics treatment.

## MATERIALS AND METHODS

Eighty patients awaiting treatment at an orthodontic specialization course of the Centro Universitário de Lavras (Lavras, MG, Brazil) were selected for participation in the study. All participants were white; 47 (58%) were female and 33 (42%) were male. The characteristics of the sample and the methodological criteria are described in a previous publication.[17]

Before starting treatment, it was performed the patch test to select patients who were allergic. Forty-two subjects (57.5%) had allergy to nickel, ranging ages from 10 to 43 years and were randomly divided in 2 groups: group I (conventional braces: n=21) and Group II (nickel-free braces: n =21).

Clinical gingival characteristics (color, volume, and bleeding) were assessed. For the evaluation, the Löe Gingival Index was used.[18]

Assessments of gingival status were carried out by a single, blinded, duly calibrated examiner (Kappa = 0.88), before starting treatment ($T_0$), followed at regular 3-month intervals for 12 months (total of five evaluations: $T_0$, $T_1$, $T_2$, $T_3$, $T_4$) with braces in place. The gingival index was used every 3 months for a total period of 12 months. However, only $T_3$ was used for blood correlations, since it coincided with the blood-sample collection.

All participants underwent a full blood test, assessment of total immunoglobin E (IgE), determination of the amount of circulating nickel in the blood, before starting treatment ($T_0$) and throughout the orthodontic treatment ($T_3$). For the examinations, 6 mL of blood was vacuum collected (vacuo-time system) from all patients after they fasted for 8 hours. For the blood count, 3mL of blood in a vacuum tube with the EDTA anticoagulant was analyzed on an automated hematology analyzer, by using the ABX

Micros CRP device (OT-CT-OS-CS, France). The differential count of blood cells was performed through a blood smear without anticoagulant, stained with Single Prov stain (NewProv - eosin methylene blue solution of cyclohexadiene to 0.1%), viewed under a microscope and read, with the aim of immersion and platelet count. For the evaluation of total IgE, 1 mL of serum without anticoagulant was analyzed by using the chemoluminescence method on the Inmulite equipment. Two mL of blood was used to assess the amount of circulating nickel, collected in a trace tube for atomic absorption spectrophotometry (graphite kiln with Zeeman corrector). In the blood count, analyses were performed of leukocytes, basophils, eosinophils, myelocytes, metamyelocytes, bands, neutrophils, lymphocytes, and monocytes.

Feces examinations were performed on all participants to determine parasitic infestations (helminth eggs and larvae, protazoon cysts) that might affect the white blood cell count, especially the number of eosinophils. The feces were collected in a sterile container, and patients were instructed to avoid contamination, using the method of Hoffman, Pons, and Janer[19] with centrifugation and spontaneous sedimentation of recent feces with no conservatives. The material was placed on a slide and assessed under a microscope (Alphaphot, 2Y52, Nikon, New York, NY). All laboratory examinations were performed by a duly trained pharmacist-biochemist. Patients did not had verminosis.

Data were analyzed using the Mann-Whitney test, paired and unpaired t test, Wilcoxon, Pearson and Spearman correlation ($p \leq .05$). The study received approval from the Research Ethics Committee of the Lavras University Center, under process number 0015.0189.000-10. The legal guardians and the adolescents signed informed consent forms authorizing participation in the study.

## RESULTS

There was a 57.5% prevalence of nickel allergy (42 individuals), 67% (28) of which occurred in female patients and 33% (14) in male patients. Five patients withdrew from the study (1 patient in the group treated with conventional braces and 4 patients in nickel free group). Tables I and II show the comparison of blood components in both groups at times $T_0$ and $T_3$ (before the start of treatment and 9 months after onset). Only data that comprise white blood cells are presented, since red blood cells are not related to immune response. There were no significant differences between groups for the moment $T_0$ ($p>.05$) and $T_3$, only the segmenteds targeted significant difference, with a larger value for the conventional group compared to nickel-free ($p\leq.05$).

Table III displays the intragroup findings in moments $T_0$ and $T_3$. For the conventional group, there was significant difference for the variables basophils and nickel (which increased in value), eosinophils and IgE (with the reduction in value); for the group Nickel free, basophils and nickel (increased value) ($p\leq.05$). Additionally, tables IV and V show the correlation between the concentration of nickel with the variables IgE, basophils and eosinophils and gengival index between times $T_0$ and $T_3$ with the number of bands and segmented in both groups. There was no significant correlation ($p>.05$).

## DISCUSSION

This study aimed to evaluate the relationship between gingival status and blood parameters in allergic patients treated with convencional and nickel free braces before e during orthodontic treatments. The type and duration of oral exposure to nickel alloys

capable of initiating an adverse reaction is controversial. Metal ions in the saliva can be swallowed prior to causing a reaction or may be absorbed in the mouth, and the amount of nickel released from the dental alloys is significantly lower than that consumed as part of food ingestion.[19,20]

Nickel is known to potentially cause allergic reactions in the oral cavity[6]. However, we found no correlation between the circulating nickel levels and IgE quantification for either conventional or nickel free braces (Table 4), although IgE play an important role in the pathogenesis of some inflammatory and allergic reactions.[21] This indicates that nickel is potentially not capable of influencing the peak production of IgE.[22] Hypersensitive reactions to nickel over a long period of time are only likely to occur with prior sensitization from non-dental contact, which is rare, and small doses of nickel may induce tolerance to this allergen.[20] With orthodontic braces, continuous exposure within the oral cavity may be a pivotal factor supporting tolerance in individuals who have not been previously sensitized.[23] Few allergic reactions seem to be associated with the use of allergens in dental materials because the oral mucosal membranes are less reactive than the skin.[24] Another factor that could explain the absence of allergic reactions in the oral cavity is the development of tolerance to nickel.[25]

In the present study, there was an increase in basophils and nickel in both groups, and a decrease in IgE which indicates the installation of a $T_H2$ immune response. These results corroborate findings described by Niiyama et al., who also assessed the systemic response produced by nickel.[26,27] A number of studies also report a nickel-produced response with a predominance of $T_H1$ $CD4^+$ T cells due to the presence of interferon-γ, but the balance generally tends to favor the expression of $T_H2$

cells and inhibit other subpopulations.[6,28-30] However, one explanation for the results is demonstrated by the IgE high coefficient of variation found.

The study carried out by Pereira et al.[31] did not found histopathological differences between groups with nickel-free and conventional braces in rats. However, an increase was found in the total number of leukocytes in the group with nickel brackets, when compared with the nickel-free brackets. The increased number of leukocytes in the circulation is expected during adaptive responses, which in this case, are stimulated by the presence of nickel.[32] When this response becomes exaggerated and there is the participation of T lymphocytes, macrophages, neutrophils and monocytes, has been the conditiontype hypersensitivity IV.[33] However, this study evaluated histopathological features in rats, which make comparisons difficult when extrapolating to humans.

It would therefore be plausible to presuppose that the continuous release of small amounts of nickel to the epithelium could be an initiating factor of gingival over-growth induced by orthodontic therapy[5]. Other studies also point to the positive characteristics of nickel-free braces in terms of the release of nickel and allergic manifestations.[15,34]

Analyses of cytokine production by Ni-specific T cells have demonstrated a mixed Th1 and Th2 cytokine profile in both T-cell clones and peripheral blood mononuclear cells.[35,36] A similar result occurred regarding the number of lymphocytes, which also increased between evaluation times, but this increase did not achieve statistical significance. This may be explained by the fact that individuals allergic to nickel have a small number or lack of specific suppressor T cells, which regulate the number of leukocyte populations.[37] Nickel suppresses NFAT signaling, which leads to the suppression of expression of a variety of cytokines and chemokines that are induced

by engagement of the T-cell-receptor. This suppression is mainly caused by the blocking effects of nickel on CRAC channels that are coupled with the engagement of the T-cell-receptor. Since humans are constantly exposed to nickel-containing products, it is important to be aware of all potential risks of nickel to our health.[38]

## CONCLUSIONS

Individuals allergic to nickel treated with nickel-free braces had lower blood disorders than individuals treated with conventional braces, but still within the normal range.

## Acknowledgements

This study was supported by the National Council for Scientific and Technological Development (CNPq) and the State of Minas Gerais Research Foundation (FAPEMIG), Brazil.

## REFERENCES

1- Kapsenberg, ML. Chemicals and proteins as allergens and adjuvants. Toxicol Lett 1996;86:79-83.

2- Wataha IC, Sun ZL, Hanks CT, Fang DN. Effect of Ni ions on expression of intercellular adhesion molecule 1 by endothelial cells. J Biomed Mater Res 1997;36:145-51.

3- Wataha JC, Lockwood PE, Marek M, Ghazi M. Ability of Ni containing biomedical alloys to activate monocytes and endothelial cells in vitro. J Biomed Mater Res 1999;45:251-7.

4- Menezes LM, Campos LC, Quintão CC, Bolognese AM. Hypersensitivity to metals in orthodontics. Am J Orthod Dentofacial Orthop 2004;126:58-64.

5- Gursoy UK, Sokucu O, Uitto VJ, Aydin A, Demirer S, Toker H, Erdem O, Sayal A. The role of nickel accumulation and epithelial cell proliferation in orthodontic treatment induced overgrowth. Eur J Orthod 2007;29:555-58.

6- Marigo M, Nouer DF, Genelhu MC, Malaquias LC, Pizziolo VR, Costa AS, Martins-Filho OA, Alves-Oliveira LF. Evaluation of immunologic profile in patients with nickel sensitivity due to use of fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 2003;124:46-52.

7- Pazzini CA, Júnior GO, Marques LS, Pereira CV, Pereira LJ. Prevalence of nickel allergy and longitudinal evaluation of periodontal abnormalities in orthodontic allergic patients. Angle Orthod 2009;79:922-7.

8- Pazzini CA, Pereira LJ, Marques LS, Generoso R, de Oliveira Jr G. Allergy to nickel in orthodontic patients: clinical and histopathologic evaluation. Gen Dent 2010;58:58-61.

9- Pazzini CA, Pereira LJ, Carlos RG, de Melo GE, Zampini MA, Marques LS. Nickel: Periodontal status and blood parameters in allergic orthodontic patients. Am J Orthod Dentofacial Orthop 2011;139:55-9.

10- Pazzini CA, Marques LS, Pereira LJ, Corrêa-Faria P, Paiva SM. Allergic reactions and nickel-free braces: A systematic review. Braz Oral Res 2011;25:85-90.

11- Johansson K, Kerosuo H, Lammintausta K. Nickel sensitization in orthodontically treated and non-treated female adolescents. Contact Dermatitis 2011;64:132-37.

12- Garcia-Gavin J, Armario-Hita JC, Fernández-Redondo V, Fernández-Vozmediano JM, Sánchez-Pérez J, Silvestre JF, et al. Nickel allergy in Spain needs active intervention. Contact Dermatitis 2011;64:289-302.

13- Schmalz G, Garhammer P. Biological interactions of dental cast alloys with oral tissues Dent Mater 2002;18:396-406.

14- Hamula DW, Hamula W, Sernetz F. Pure titanium orthodontic brackets. J Clin Orthod 1996;30:140-4.

15- Rahilly G, Price N. Nickel allergy and orthodontics. J Orthod 2003;30:171-4

16- Pantuzo MCG, Zenóbio EG, Marigo HA, Zenóbio MAF. Hypersensitivity to conventional and to nickel-free orthodontic brackets. Braz Oral Res 2007;21:298-302.

17- Pazzini CA, Marques LS, Ramos-Jorge ML, Júnior GO, Pereira LJ, Paiva SM. Longitudinal assessment of periodontal status in patients with nickel allergy treated with conventional and nickel-free braces. Angle Orthod 2012;82:653-7.

18- Loe H. The Gingival Index, the Plaque Index and Retention Index systems. J Periodontol 1967;38:610-6.

19- Park HY, Shearer TR. In vitro release of nickel and chromium from simulated orthodontic appliances. Am J Orthod 1983;84:156-9.

20- Setcos JC, Babaei-Mahani A, Silvio LD, Mjor IA, Wilson NH. The safety of nickel containing dental alloys. Dent Mater 2006;22:1163-8.

21- Jafarzadeh A, Sadeghi M, Karam GA, Vazirinejad R.Salivary IgA and IgE levels in healthy subjects: relation to age and gender. Braz Oral Res 2010;24:21-7.

22- Kulak Y, Arikan A. Effect of dental base metal alloys on IgE levels and some blood parameters. J Oral Rehabil 1997;24:749-754.

23- Van Hoogstraten IM, Andersen KE, Von Blomberg BM, Boden D, Bruynzeel DP, Burrows D, Camarasa JG, et al. Reduced frequency of nickel allergy upon oral nickel contact at an early age. Clin Exp Immunol 1991;85:441-5.

24- Haberman A, Pratt M, Storrs T. Contact dermatitis from beryllium in dental alloys. Contact Dermatitis 1993;28:157-62.

25- House K, Sernetz F, Dymock D, Sandy JR, Ireland AJ. Corrosion of orthodontic appliances: should we care? Am J Orthod Dentofacial Orthop 2008;133:584-92.

26- Chakir H, Wang H, Lefebvre DE, Webb J, Scott FW. T-bet/GATA-3 ratio as a measure of the Th1/Th2 cytokine profile in mixed cell populations: predominant role of GATA-3. J Immunol Methods 2003;278:157-169.

27- Niiyama S, Tamauchi H, Amoh Y, Terashima M, Matsumura Y, Kanoh M, Habu S, Komotori J, Katsuoka K. Th2 immune response plays a critical role in the development of nickel-induced allergic contact dermatitis. Int Arch Allergy Immunol 2010;153:303-14.

28- Minang JT, Areström I, Troye-Blomberg M, Lundeberg L, Ahlborg N. Nickel, cobalt, chromium, palladium and gold induce a mixed Th1- and Th2- type cytokine response in vitro in subjects with contact allergy to the respective metals. Clin Exp Immunol 2006a;146:417-26.

29- Minang JT, Areström I, Zuber B, Jönsson G, Troye-Blomberg M, Ahlborg N. Nickel-induced IL-10 down-regulates Th1- but not Th2-type cytokine responses to the contact allergen nickel. Clin Expl Immunol 2006b;143:494-502.

30- Schwenk M, Sackb U, Esserc C, Klein R. Diagnostic relevance of the determination of lymphocyte subpopulations in environmental medicine. Int J Hyg Environ Health 2007;210:177-98.

31- Pereira CV, Kaminagakura E, Bonan PR, Bastos RA, Pereira LJ. Cellular, humoral, and histopathologic analysis in rats implanted with orthodontic nickel brackets. Angle Orthod 2008;78:114-9.

32- Moser M, Leo O. Key concepts in immunology. Vaccine 2010;28:2-13.

33- Cruvinel WM, Júnior DM, Araújo JAP, Catelan TTT, Souza AWS, Silva NP. Molecular and cellular aspects of innate immunity. Rev Bras Reumatol 2010;50:434-61.

34- Sfondrini MF, Cacciafesta V, Maffia E, Massironi S, Scribante A, Alberti G, et al. Chromium release from new stainless steel, recycled and nickel-free orthodontic brackets. Angle Orthod 2009;79:361-7.

35- Jakobson E, Masjedi K, Ahlborg N, Lundeberg L, Karlberg AT, Scheynius A. Cytokine production in nickel-sensitized individuals analysed with enzyme-linked immunospot assay: possible implication for diagnosis. Br J Dermatol 2002;147:442-9.

36- Rustemeyer T, Von Blomberg BM, Van Hoogstraten IM, Bruynzeel DP, Scheper RJ. Analysis of effector and regulatory immune reactivity to nickel. Clin Exp Allergy 2004;34:1458-66.

37- Cavani A. Breaking tolerance to nickel. Toxicology 2005;209:119-21.

38- Saito R, Hirakawa S, Ohara H, Yasuda M, Yamazaki T, Nishii S, Aiba S. Nickel differentially regulates NFAT and NF-κB activation in T cell signaling. Toxicol Appl Pharmacol 2011;245:245-55.

Table I: Comparison of blood components from patients using conventional and nickel free braces: 1 month before starting treatment ($T_0$)

| Group | Mean | Standard desviation | Median | (25th,75th) | p-value |
|---|---|---|---|---|---|
| Leukocytes(mil/mm$^3$) | | | | | |
| convencional | 6025.00 | 1771.37 | 5750.00 | 4625.00,6775.00 | 0.354[A] |
| níquel free | 5450.58 | 1948.46 | 5400.00 | 4250.00,6850.00 | |
| Basophils(mil/mm$^3$) | | | | | |
| convencional | 10.00 | 0.73 | 10.00 | 0.00,20.00 | 0.272[B] |
| níquel free | 6.47 | 9.31 | 0.00 | 0.00,10.00 | |
| Eosinophils(mil/mm$^3$) | | | | | |
| convencional | 367.50 | 270.53 | 320.00 | 160.00,550.00 | 0.860[A] |
| níquel free | 381.82 | 209.51 | 340.00 | 165.00,585.00 | |
| Bands(mil/mm$^3$) | | | | | |
| convencional | 37.27 | 100.53 | 0.00 | 0.00,0.00 | 0.902[B] |
| níquel free | 29.41 | 98.51 | 0.00 | 0.00,0.00 | |
| Segmenteds(mil/mm$^3$) | | | | | |
| convencional | 5358.50 | 913.24 | 5365.00 | 4637.50,6160.00 | 0.170[A] |
| níquel free | 4910.58 | 1032.80 | 5100.00 | 4510.00,5485.00 | |
| Lymphocytes(mil/mm$^3$) | | | | | |
| convencional | 3528.50 | 846.24 | 3525.00 | 2997.50,4032.50 | 0.225[A] |
| níquel free | 3897.05 | 968.00 | 3790.00 | 3135.00,4280.00 | |
| Monocytes(mil/mm$^3$) | | | | | |
| convencional | 652.50 | 224.82 | 700.00 | 532.50,797.50 | 0.489[A] |
| níquel free | 599.41 | 236.18 | 630.00 | 500.00,755.00 | |
| IgE(UI/mL) | | | | | |
| convencional | 479.55 | 773.79 | 102.50 | 37.47,653.25 | 0.532[B] |
| níquel free | 469.56 | 545.75 | 240.00 | 36.85,851.00 | |
| Nickel(mcg/L) | | | | | |
| convencional | 1.24 | 1.26 | 0.70 | 0.43,1.88 | 0.542[B] |
| níquel free | 0.80 | 0.82 | 0.71 | 0.10,1.37 | |

[A] T test; [B] Mann-Whitney test

Table II: Comparison of blood components from patients using conventional and nickel free braces: 9 months after starting treatment ($T_3$)

| Group | Mean | Standard desviation | Median | (25th,75th) | p-value |
|---|---|---|---|---|---|
| Leukocytes (mil/mm$^3$) | | | | | |
| convencional | 5920.00 | 2244.34 | 6000.00 | 4275.00,7600.00 | 0.978[A] |
| níquel free | 5900.00 | 1947.11 | 6500.00 | 4850.00,7300.00 | |
| Basophils (mil/mm$^3$) | | | | | |
| convencional | 26.84 | 17.65 | 25.00 | 10.00,40.00 | 0.957[A] |
| níquel free | 26.47 | 23.16 | 20.00 | 0.00,50.00 | |
| Eosinophils (mil/mm$^3$) | | | | | |
| convencional | 271.94 | 191.36 | 205.00 | 162.5,297.5 | 0.680[B] |
| níquel free | 294.35 | 185.80 | 260.00 | 150.00,430.00 | |
| Bands(mil/mm$^3$) | | | | | |
| convencional | 31.57 | 115.72 | 0.00 | 0.00,0.00 | 0.597[B] |
| níquel free | 0.00 | 0.00 | 0.00 | 0.00,0.00 | |
| Segmenteds (mil/mm$^3$) | | | | | |
| convencional | 5297.89 | 982.18 | 5175.00 | 4622.50,6015.00 | **0.040**[A] |
| níquel free | 4668.82 | 756.39 | 4710.00 | 4230.00,5070.00 | |
| Lymphocytes (mil/mm$^3$) | | | | | |
| convencional | 3552.10 | 906.21 | 3410.00 | 2792.50,4322.50 | 0.082[A] |
| níquel free | 4102.35 | 937.08 | 3960.00 | 3520.00,4785.00 | |
| Monocytes (mil/mm$^3$) | | | | | |
| convencional | 659.47 | 262.98 | 765.00 | 605.00,825.00 | 0.739[B] |
| níquel free | 547.88 | 342.48 | 700.00 | 102.00,850.00 | |
| IgE(UI/mL) | | | | | |
| convencional | 370.74 | 646.59 | 85.95 | 18.55,435.75 | 0.568[B] |
| níquel free | 414.99 | 531.85 | 206.00 | 41.00,648.00 | |
| Nickel (mcg/L) | | | | | |
| convencional | 2.05 | 0.79 | 1.74 | 1.49,2.39 | 0.194[B] |
| níquel free | 1.77 | 0.78 | 1.51 | 1.40,2.15 | |

[A] T test; [B] Mann-Whitney test

Table III: Comparison between blood components within each group (conventional and nickel free) between times ($T_0$ and $T_3$)

| Components | Conventional Group | | | Nickel free Group | | |
|---|---|---|---|---|---|---|
| | Exam 1 (mean±dp) | Exam 2 (mean±dp) | p-value | Exam 1 (mean±dp) | Exam 2 (mean±dp) | p-value |
| **Leukocytes** | 6025.00±1771.37 | 5920.00±2244.34 | 0.864 | 5450.58±1948.46 | 5900.00±1947.11 | 0.063[A] |
| **Basophils** | 10.00±0.73 | 26.84±17.65 | **0.003** | 6.47±9.31 | 26.47±23.16 | **0.001**[A] |
| **Eosinophils** | 367.50±270.53 | 271.94±191.36 | **0.005** | 381.82±209.51 | 294.35±185.80 | 0.208[B] |
| **Bands** | 37.27±100.53 | 31.57±115.72 | 1.000 | 29.41±98.51 | 0.00±0.00 | 0.500[B] |
| **Segmenteds** | 5358.50±913.24 | 5297.89±982.18 | 0.920 | 4910.58±1032.80 | 4668.82±756.39 | 0.204[A] |
| **Lymphocytes** | 3528.50±846.24 | 3552.10±906.21 | 0.978 | 3897.05±968.00 | 4102.35±937.08 | 0.528[A] |
| **Monocytes** | 652.50±224.82 | 659.47±262.98 | 0.395 | 599.41±236.18 | 547.88±342.48 | 0.890[B] |
| **IgE** | 479.55±773.79 | 370.74±646.59 | **0.016** | 469.56±545.75 | 414.99±531.85 | 0.306[B] |
| **Nickel** | 1.24±1.26 | 2.05±0.79 | **0.024** | 0.80±0.82 | 1.77±0.78 | **0.004**[A] |

[A] Paired t test; [B] Wilcoxon test

Table IV: Correlation between the concentration of Nickel with variables IgE, Basophils and Eosinophils at $T_0$ and $T_3$

| **Exam 1** | | **IgE** | **Basophils** | **Eosinophils** |
|---|---|---|---|---|
| Nickel – conventional | p | 0.657 | 0.999 | 0.163 |
| | r | -0.106 | 0.000 | -0.325 |
| Nickel – nickel free | p | 0.764 | 0.726 | 0.928 |
| | r | 0.078 | -0.091 | 0.023 |
| **Exam 2** | | **IgE** | **Basophils** | **Eosinophils** |
| Nickel – conventional | p | 0.301 | 0.628 | 0.636 |
| | r | -0.250 | 0.119 | 0.116 |
| Nickel – nickel free | p | 0.499 | 0.727 | 0.423 |
| | r | 0.176 | -0.091 | -0.208 |

Pearson Correlations
p = p value or descriptive level
r = linear correlation coefficient

Table V: Correlation between GI examinations with the variables Bands and Segmenteds at $T_0$ and $T_3$

| **GI $T_0$** | | **Bands** | **Segmenteds** |
|---|---|---|---|
| conventional | p | 0.947 | 0.807 |
| | r | 0.015 | 0.056 |
| nickel free | p | 0.936 | 0.824 |
| | r | 0.019 | -0.055 |
| **GI $T_3$** | | **Bands** | **Segmenteds** |
| conventional | p | 0.715 | 0.787 |
| | r | -0.087 | -0.065 |
| nickel free | p | 0.065 | 0.111 |
| | r | 0.471 | 0.397 |

Spearman Correlations
p = p value or descriptive level
r = linear correlation coefficient

# CONSIDERAÇÕES FINAIS

## CONSIDERAÇÕES FINAIS

A partir de uma avaliação crítica dos estudos encontrados foi possível constatar que o níquel é capaz de promover alterações gengivais e sanguíneas em pacientes alérgicos. Esse problema torna-se crítico principalmente quando se considera o aumento da demanda pelo tratamento ortodôntico. Recentemente, estudos de natureza prospectiva, bem como de revisão sistemática da literatura, forneceram importantes informações para uma melhor compreensão da alergia ao níquel em pacientes ortodônticos (Kolokitha et al., 2008; Pazzini et al., 2009, 2011).

Piores condições gengivais foram observadas ao longo do tratamento ortodôntico em indivíduos alérgicos ao níquel, quando comparados com indivíduos não alérgicos ao níquel, sugerindo um efeito cumulativo do níquel associado a alterações gengivais (Pazzini et al., 2009).

Braquetes níquel free tem ganhado popularidade como uma alternativa viável para pacientes ortodônticos alérgicos ao níquel (Pantuzo et al., 2007; Kolokitha et al., 2008; Pazzini et al. 2011, 2012). Indivíduos com alergia ao níquel apresentam melhor saúde gengival quando tratados com braquetes níquel free do que com braquetes convencionais (Pazzini et al., 2012).

No momento da anamnese, é importante indagar ao paciente sobre sua história prévia de sensibilidade a metais. Em pacientes alérgicos, o profissional deve optar por materiais biocompatíveis, como por exemplo, braquetes cerâmicos ou níquel free. Além disso, é necessário enfatizar com o paciente a importância de uma atenção especial com a higiene bucal, para também minimizar a corrosão dos metais, principalmente em pacientes apresentando problemas gengivais e periodontais.

Estudos futuros devem incluir as taxas de cromo e cobalto, que também são substâncias presentes em materiais ortodônticos e podem estar superestimando os efeitos do níquel. Além disso, estudos com abordagem molecular podem oferecer respostas ainda mais específicas sobre os mecanismos associados à alergia ao níquel em pacientes ortodônticos.

# REFERÊNCIAS
# Considerações Iniciais e Finais
# Material e Métodos

## REFERÊNCIAS

1. Barrett RD, Bishara SE, Quinn JK. Biodegradation of orthodontic appliances. Part I. Biodegradation of nickel and chromium in vitro. Am J Orthod Dentofacial Orthop 1993;103:8-14.

2. Bishara SE, Barrett RD, Selim MI. Biodegradation of orthodontic appliances. Part II. Changes in the blood level of nickel. Am J Orthod Dentofacial Orthop 1993;103:115-9.

3. Bordji K, Jouzeau JY, Mainard D, Payan E, Delagoutte JP, Netter P. Evaluation of the effect of three surface treatments on the biocompatibility of 316L stain-less steel using human differentiated cells. Biomaterials 1996;17:491-500.

4. Eliades T, Athanasiou AE. In vivo aging of orthodontic alloys: implications for corrosion potential, nickel release, and biocompatibility. Angle Orthod 2002;72:222-37.

5. Evans TJ, Durning P. Aligning archwires, the shape of things to come? A fourth and fifth phase of force delivery. Br J Orthod 1996;23:269-75.

6. Fisher AA, Menné T, Johansen JD. Dose per unit area-a study of elicitation of nickel allergy. Contact Dermatitis 2007;56:255-61.

7. Fors R, Persson M. Nickel in dental plaque and saliva in patients with and without orthodontic appliances. Eur J Orthod 2006;28:292-7.

8. GBEDC (Grupo brasileiro de estudos em dermatite de contato). Estudo multicêntrico para elaboração de uma bateria padrão brasileira de testes de contato. Bras Dermatol 2000;75:147-56.

9. Gürsoy S, Acar AG, Seşen C. Comparison of metal release from new and recycled bracket-archwire combinations. Angle Orthod 2005;75:92-4.

10. Hamilton WA. Bioenergetics of sulphate-reducing bacteria in relation to their environmental impact. Biodegradation 1998;9:201-12.

11. Hamula DW, Hamula W, Sernetz F. Pure titanium orthodontic brackets. J Clin Orthod 1996;30:140-4.

12. Huang T, Yen C, Kao C. Comparison of ion release from new and recycled orthodontic brackets. Am J Orthod Dentofacial Orthop 2001;120:68-75.

13. Janson GR, Dainese EA, Consolaro A, Woodside DG, Freitas MR. Nickel hypersensitivity reaction before, during and after orthodontic therapy. Am J Orthod Dentofacial Orthop 1998;113:655-60.

14. Johansson K, Kerosuo H, Lammintausta K. Nickel sensitization in orthodontically treated and non-treated female adolescents. Contact Dermatitis 2011;64:132-7.

15. Kao CT, Ding SJ, Min Y, Hsu TC, Chou MY, Huang TH. The cytotoxicity of orthodontic metal bracket immersion media. Eur J Orthod 2007;29:198-203.

16. Kolokitha OE, Kaklamanos EG, Papadopoulos MA. Prevalence of nickel hypersensitivity in orthodontic patients: a meta-analysis. Am J Orthod Dentofacial Orthop 2008;134:722-34.

17. Levrini L, Lusvardi G, Gentile D. Nickel ions release in patients with fixed orthodontic appliances. Minerva Stomatol 2006;55:115-21.

18. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159-74.

19. Löe H. The Gingival Index, the Plaque Index and the Retention Index Systems. J Periodontol 1967;38:610-6.

20. Marigo M, Nouer DF, Genelhu MC, Malaquias LC, Pizziolo VR, Costa AS, Martins-Filho OA, Alves-Oliveira LF. Evaluation of immunologic profile in patients with nickel sensitivity due to use of fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 2003;124:46-52.

21. Menezes LM, Campos LC, Quintão CC, Bolognese AM. Hypersensitivity to metals in orthodontics. Am J Orthod Dentofacial Orthop 2004;126:58-64.

22. Minang JT, Areström I, Zuber B, Jönsson G, Troye-Blomberg M, Ahlborg N. Nickel-induced IL-10 down-regulates Th1 but not Th2-type cytokine responses to the contact allergen nickel. Clin Exp Immunol 2006;143:494-502.

23. Ministério da Saúde, Secretaria de políticas de Saúde, Coordenação Nacional de DST e AIDS. Controle de infecções e a prática odontológica em tempos de AIDS: manual de condutas. Brasília: Ministério da Saúde, 2000:118p.

24. Organização Mundial de Sáude – OMS. Levantamentos básicos em saúde bucal. 4ed. São Paulo: Santos; 1999. 66p.

25. Pantuzo MCG, Zenóbio EG, Marigo HA, Zenóbio MAF. Hypersensitivity to conventional and to nickel-free orthodontic brackets. Braz Oral Res 2007;21:298-302.

26. Park HY, Shearer TR. In vitro release of nickel and chromium from simulated orthodontic appliances. Am J Orthod 1983;84:156-9.

27. Platt JA, Guzman A, Zuccari A, Thornburg DW, Rhodes BF, Oshida Y, Moore BK. Corrosion behavior of 2205 duplex stainless steel. Am J Orthod Dentofacial Orthop 1997;112:69-79.

28. Pazzini CA, Júnior GO, Marques LS, Pereira CV, Pereira LJ. Prevalence of nickel allergy and longitudinal evaluation of periodontal abnormalities in orthodontic allergic patients. Angle Orthod 2009;79:922-7.

29. Pazzini CA, Pereira LJ, Marques LS, Generoso R, de Oliveira G Jr. Allergy to nickel in orthodontic patients: clinical and histopathologic evaluation. Gen Dent 2010;58:58-61.

30. Pazzini CA, Pereira LJ, Generoso RG, Brito-Melo GEA, Zampini MA, Marques LS. Nickel: periodontal status and blood parameters in allergic orthodontic patients. Am J Orthod Dentofacial Orthop 2011;139:55-59.

31. Pazzini CA, Marques LS, Pereira LJ, Corrêa-Faria P, Paiva SM. Allergic reactions and nickel-free braces: a systematic review. Braz Oral Res 2011;25:85-90.

32. Pazzini CA, Marques LS, Ramos-Jorge ML, Júnior GO, Pereira LJ, Paiva SM. Longitudinal assessment of periodontal status in patients with nickel allergy treated with conventional and nickel-free braces. Angle Orthod 2012;82:653-57.

33. Peltonen L. Nickel sensitivity in the general population. Contact Dermatitis 1979;5:27-32.

34. Pereira CV, Kaminagakura E, Bonan PR, Bastos RA, Pereira LJ. Cellular, humoral, and histopathologic analysis in rats implanted with orthodontic nickel brackets. Angle Orthod 2008;78:114-9.

35. Pereira MG. Epidemiologia: teoria e prática. 4. ed. Rio de Janeiro: Guanabara Koogan, 2000; 273.

36. Rahilly G, Price N. Nickel allergy and orthodontics. J Orthod 2003;30:171-4.

37. Rietschel RL, Fowler JF. Fisher´s contact dermatitis. 6th ed. Baltimore: Lippincott Willians & Wilkins; 2001.

38. Santucci B, Cannistraci C, Cristaudo A, Picardo M. Interaction of metals in nickel-sensitive patients. Contact Dermatitis 1993;29:251-3.

39. Saito R, Hirakawa S, Ohara H, Yasuda M, Yamazaki T, Nishii S, Aiba S.

Source Nickel differentially regulates NFAT and NF-κB activation in T cell signaling. Toxicol Appl Pharmacol 2011;254:245-55.

40. Setcos JC, Babaei-Mahani A, Silvio LD, Mjör IA, Wilson NH. The safety of nickel containing dental alloys. Dent Mater 2006;22:1163-8.

41. Silvennoinen-Kassinen S, Ikäheimo I, Karvonen J, Kauppinen M, Kallioinen M. Mononuclear cell subsets in the nickel-allergic reaction in vitro and in vivo. J Allergy Clin Immunol 1992;89:794-800.

42. Sória ML, Menezes L, Dedavid B, Pires M, Rizzatto S, Filho LCC. In vitro nickel release from metallic orthodontic brackets. Rev Dent Press Ortodon Ortop Facial 2005;10:87-96.

43. Spiewak R, Moed H, Von Blomberg BM, Bruynzeel DP, Scheper RJ, Gibbs S, Rustemeyer T. Allergic contact dermatitis to nickel: modified in vitro test protocols for better detection of allergen-specific response. Contact Dermatitis 2007;56:63-9.

44. World Health Organization (WHO). Calibration of examiners for oral health epidemiology surveys. Technical Report. Geneva: WHO; 1993.

# APÊNDICES

## APÊNDICE A Termo de Consentimento Livre e Esclarecido

Prezados Senhores Pais/Responsáveis,

Sou Camila Alessandra Pazzini, aluna de doutorado da Faculdade de Odontologia da Universidade Federal de Minas Gerais (UFMG). Estamos desenvolvendo um trabalho de pesquisa sobre a alergia ao níquel, uma substância que está presente no aparelho ortodôntico fixo.

Esse trabalho será realizado na clínica de Ortodontia, da Faculdade Unilavras, onde seu filho (a) estará em tratamento. Para a realização dessa pesquisa será feito um teste de alergia. Esse teste é feito colando um pequeno adesivo nas costas do seu filho, por 48 horas. Depois de removido o adesivo saberemos se o seu filho apresenta alergia. O exame da alergia não dói, aparecendo às vezes uma vermelhidão no local e uma pequena coceira. Tirando o adesivo, a vermelhidão desaparece. Será feito também, a cada 3 meses, limpeza nos dentes. Antes de colocar o aparelho, 9 meses depois da colocado e após a remoção do aparelho serão feitos exames de sangue para avaliação geral do sangue do seu filho. O exame de sangue terá o pequeno desconforto da picada da agulha no braço de seu filho, da mesma forma como acontece com qualquer outro exame de sangue. O sangue será colhido com agulha descartável e o profissional usará luvas descartáveis e todo o material de proteção individual como avental, gorro, óculos e máscara descartável. Serão utilizados 2 tipos de aparelho: um normal e um com menor quantidade de níquel. Os aparelhos possuem o mesmo tamanho e cor e são colocados da mesma maneira nos dentes de seu filho. Todas as crianças entrarão num sorteio para a escolha do aparelho.

Vocês têm o direito de participar ou não e podem desistir de participar da pesquisa a qualquer momento. Não haverá nenhum custo financeiro para vocês. Garantimos que o nome do seu filho (a) não aparecerá em nenhum momento.

Caso você esteja de acordo com a participação de seu (ua) filho (a) na pesquisa, precisamos da sua autorização.

Estamos à sua disposição para maiores esclarecimentos pelos telefones (35)98042133 ou (35)38219530 e ainda pelo e-mail camilapazzini@hotmail.com.

Esta pesquisa será enviada para o Comitê de Ética em Pesquisa da UFMG (telefone: 3409-4592).

Eu,_____, responsável por _____, de _____ anos de idade, declaro ter sido devidamente esclarecido (a) e autorizo a participação de meu filho (a) na pesquisa "Alterações sanguíneas e periodontais em pacientes alérgicos ao níquel sob tratamento ortodôntico com braquetes convencionais e níquel free: ensaio clínico randomizado".

_____

**Camila Alessandra Pazzini**

**Pesquisadora responsável**

**Telefones: (35) 38219530 / (35) 98042133**

Belo Horizonte, _____ de _____ de ____.

## APÊNDICE B Termo de Consentimento Livre e Esclarecido

Prezado paciente menor de 18 anos,

Sou Camila Alessandra Pazzini, aluna de doutorado da Faculdade de Odontologia da Universidade Federal de Minas Gerais (UFMG). Estamos desenvolvendo um trabalho de pesquisa sobre a alergia ao níquel, uma substância que está presente no aparelho ortodôntico fixo.

Esse trabalho será realizado na clínica de Ortodontia, da Faculdade Unilavras, onde você estará em tratamento. Para a realização dessa pesquisa será feito um teste de alergia. Esse teste é feito colando um pequeno adesivo nas suas costas, onde ele deve ficar por dois dias. Depois de dois dias vamos remover o adesivo e saber se você tem alergia ou não. O exame da alergia não dói. Ás vezes fica um pouco vermelho no local e coça um pouco. Tirando o adesivo, a vermelhidão desaparece. Vamos também fazer limpeza nos seus dentes, a cada 3 meses. Antes de colocar o aparelho, 9 meses depois da colocado e após a remoção do aparelho serão feitos exames de sangue para avaliação geral do seu sangue. O exame de sangue terá o pequeno desconforto da picada da agulha no braço, da mesma forma como acontece com qualquer outro exame de sangue. O sangue será colhido com agulha descartável e o profissional usará luvas descartáveis e todo o material de proteção individual como avental, gorro, óculos e máscara descartável. Serão utilizados 2 tipos de aparelho: um normal e um com menor quantidade de níquel. Os aparelhos possuem o mesmo tamanho e cor e são colocados da mesma maneira nos seus dentes. Todas as crianças entrarão num sorteio para a escolha do aparelho.

Você tem o direito de participar ou não e pode desistir de participar da pesquisa a qualquer momento. Não haverá nenhum custo financeiro para você. Garantimos que o seu nome não aparecerá em nenhum momento.

Caso você esteja de acordo com a participação na pesquisa, precisamos da sua autorização.

Estamos à sua disposição para maiores esclarecimentos pelos telefones (35)98042133 ou (35)38219530 e ainda pelo e-mail camilapazzini@hotmail.com.

Esta pesquisa será enviada para o Comitê de Ética em Pesquisa da UFMG (telefone: 3409-4592).

Eu,_____, de _____ anos de idade, declaro ter sido devidamente esclarecido (a) e autorizo a minha participação na pesquisa "Alterações sanguíneas e periodontais em pacientes alérgicos ao níquel sob tratamento ortodôntico com braquetes convencionais e níquel free: ensaio clínico randomizado".

_____

**Camila Alessandra Pazzini**

**Pesquisadora responsável**

**Telefones: (35) 38219530 / (35) 98042133**

Belo Horizonte, _____ de _____ de ____.

# APÊNDICE C Termo de Consentimento Livre e Esclarecido

Prezado paciente maior de 18 anos,

Sou Camila Alessandra Pazzini, aluna de doutorado da Faculdade de Odontologia da Universidade Federal de Minas Gerais (UFMG). Estamos desenvolvendo um trabalho de pesquisa sobre a alergia ao níquel, uma substância que está presente no aparelho ortodôntico fixo.

Esse trabalho será realizado na clínica de Ortodontia, da Faculdade Unilavras, onde você estará em tratamento. Para a realização dessa pesquisa será feito um teste de alergia. Esse teste é feito colando um pequeno adesivo nas suas costas, onde ele deve ficar por dois dias. Depois de dois dias vamos remover o adesivo e saber se você tem alergia ou não. O exame da alergia não dói. Ás vezes fica um pouco vermelho no local e coça um pouco. Tirando o adesivo, a vermelhidão desaparece. Vamos também fazer limpeza nos seus dentes, a cada 3 meses. Antes de colocar o aparelho, 9 meses depois da colocado e após a remoção do aparelho serão feitos exames de sangue para avaliação geral do seu sangue. O exame de sangue terá o pequeno desconforto da picada da agulha no braço, da mesma forma como acontece com qualquer outro exame de sangue. O sangue será colhido com agulha descartável e o profissional usará luvas descartáveis e todo o material de proteção individual como avental, gorro, óculos e máscara descartável. Serão utilizados 2 tipos de aparelho: um normal e um com menor quantidade de níquel. Os aparelhos possuem o mesmo tamanho e cor e são colocados da mesma maneira nos seus dentes. Todas as crianças entrarão num sorteio para a escolha do aparelho.

Você tem o direito de participar ou não e pode desistir de participar da pesquisa a qualquer momento. Não haverá nenhum custo financeiro para você. Garantimos que o seu nome não aparecerá em nenhum momento.

Caso você esteja de acordo com a participação na pesquisa, precisamos da sua autorização.

Estamos à sua disposição para maiores esclarecimentos pelos telefones (35)98042133 ou (35)38219530 e ainda pelo e-mail camilapazzini@hotmail.com.

Esta pesquisa será enviada para o Comitê de Ética em Pesquisa da UFMG (telefone: 3409-4592).

Eu,_____, de _____ anos de idade, declaro ter sido devidamente esclarecido (a) e autorizo a minha participação na pesquisa "Alterações sanguíneas e periodontais em pacientes alérgicos ao níquel sob tratamento ortodôntico com braquetes convencionais e níquel free: ensaio clínico randomizado".

_____

**Camila Alessandra Pazzini**

**Pesquisadora responsável**

**Telefones: (35) 38219530 / (35) 98042133**

Belo Horizonte, _____ de _____ de ____.

# ANEXOS

ANEXO A: Parecer do Comitê de Ética em Pesquisa da UFMG

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

**Parecer nº. ETIC 0125.0.203.203-11**

**Interessado(a): Prof. Saul Martins de Paiva**
**Departamento de Odontopediatria e Ortodontia**
**Faculdade de Odontologia - UFMG**

**DECISÃO**

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 14 de junho de 2011, após atendidas as solicitações de diligência, o projeto de pesquisa intitulado **"Alterações sanguíneas e periodontais em pacientes alérgicos ao níquel sob tratamento ortodôntico com braquetes convencionais e níquel free: ensaio clínico randomizado"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

**Profa. Maria Teresa Marques Amaral**
**Coordenadora do COEP-UFMG**

*Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar – Sala 2005 – Cep:31270-901 – BH-MG*
Telefax: (031) 3409-4592 - e-mail: coep@prpq.ufmg.br

ANEXO B: Aprovação dos Comitês de Ética em Pesquisa da UFMG e UNILAVRAS

**Andamento do projeto - CAAE - 0125.0.203.203-11**

**Título do Projeto de Pesquisa**

Alterações sanguineas e periodontais em pacientes alérgicos ao níquel sob tratamento ortodôntico com braquetes convencionais e níquel free: ensaio clinico randomizado

| Situação | Data Inicial no CEP | Data Final no CEP | Data Inicial na CONEP | Data Final na CONEP |
|---|---|---|---|---|
| Aprovado no CEP | 01/04/2011 14:22:03 | 15/06/2011 09:43:05 | | |

| Descrição | Data | Documento | Nº do Doc | Origem |
|---|---|---|---|---|
| 2 - Recebimento de Protocolo pelo CEP (Check-List) | 01/04/2011 14:22:03 | Folha de Rosto | 0125.0.203.203-11 | CEPV |
| 1 - Envio da Folha de Rosto pela Internet | 01/04/2011 11:11:54 | Folha de Rosto | FR414951 | Pesquisador |
| 3 - Protocolo Aprovado no CEP | 15/06/2011 09:43:05 | Folha de Rosto | 125/11 | CEP |

**Andamento do projeto - CAAE - 0015.0.189.000-10**

**Título do Projeto de Pesquisa**

AVALIAÇÃO LONGITUDINAL DAS ALTERAÇÕES PERIODONTAIS EM PACIENTES ALÉRGICOS AO NÍQUEL SUBMETIDOS À TERAPIA ORTODÔNTICA COM BRAQUETES CONVENCIONAIS E NÍQUEL FREE

| Situação | Data Inicial no CEP | Data Final no CEP | Data Inicial na CONEP | Data Final na CONEP |
|---|---|---|---|---|
| Aprovado no CEP | 06/04/2010 14:40:51 | 24/08/2010 08:24:09 | | |

| Descrição | Data | Documento | Nº do Doc | Origem |
|---|---|---|---|---|
| 1 - Envio da Folha de Rosto pela Internet | 09/03/2010 23:07:13 | Folha de Rosto | FR323059 | Pesquisador |
| 3 - Recebimento de Protocolo pelo CEP (Check-List) | 06/04/2010 14:40:51 | Folha de Rosto | 0015.0.189.000-10 | CEP |
| 2 - Recebimento de Protocolo pelo CEP (Check-List) | 06/04/2010 14:40:16 | Folha de Rosto | 0014.0.189.000-10 | CEP |
| 4 - Protocolo Aprovado no CEP | 24/08/2010 08:24:09 | Folha de Rosto | 2 | CEP |

ANEXO C: Normas de Publicação Brazilian Dental Journal

BRAZILIAN DENTAL JOURNAL

**Scope and policy**

The **Brazilian Dental Journal** publishes Full-Length Papers, Short Communications and Case Reports, dealing with dentistry or related disciplines. Only original papers will be considered for publication. In submitting a manuscript, the authors should state in the cover letter that the material has not been published previously and is not under consideration by another journal in either electronic or printed versions.

ADDRESS FOR SUBMISSION
Brazilian Dental Journal
Fundação Odontológica de Ribeirão Preto Av. do Café, s/n
14040-904 Ribeirão Preto, SP, Brasil
Fax: +55-16-633-0999 E-mails: bdj@forp.usp.br, pecora@forp.usp.br

MANUSCRIPTS MUST BE SUBMITTED IN ENGLISH. Authors whose primary language is not English must have their manuscript reviewed by someone proficient in English. **Manuscripts accepted for publication will be submitted to the Technical Review for revision of English grammar and scientific writing and to fit the text into the Journal's standards. The cost of the Technical Review will be charged to the authors. Submission of a manuscript to BDJ implies the acceptance of these terms.** The decision of acceptance for publication relies on the Editors and is based on the recommendation of the Editorial Board and/or *ad hoc* reviewers. Manuscripts not recommended for publication will not be returned, but the authors will receive an email explaining the decision. The concepts emitted in the papers published in the BDJ are the sole responsibility of the authors, not necessarily reflecting the Editorial Board's opinion.

Form and preparation of manuscripts

**THE FOLLOWING GUIDELINES MUST BE FOLLOWED CAREFULLY.**

**General**

- The authors must submit 1 (one) hard copy of the manuscript PRINTED ON BOTH SIDES, comprising the text, tables, figure captions and figures (graphs, photographs, photomicrographs, radiographs, schematic drawings, etc). The figures can be printed on good quality plain paper.
- The hard copy of the manuscript must be submitted together with a CD-ROM containing:
    - the manuscript file in Word identical to the printed version
    - the digital files of the figures (if any) saved in TIFF format
- The manuscript must be printed on both sides of good quality plain paper and must be typed in Times New Roman 12 font, with 1.5 spacing, 2.5-cm margins at each side. **DO NOT USE** bold letters, watermarks or other resources to make the text visually attractive.
- Pages should be numbered consecutively, starting with the title page.
- Full-length manuscripts are assembled in the following sections:

    1) Title Page
    2) Summary and Key Words
    3) Introduction; Material and Methods; Results; Discussion
    4) Summary in Portuguese (an item necessary for Latin American Indexing Services that will be provided for non-Brazilian authors by the Journal)
    5) Acknowledgements (if any)
    6) References
    7) Tables
    8) Figure captions
    9) Figures

- All titles of sections (Introduction, Material and Methods, etc) must be capitalized in regular font type (not bold).
- Results and Discussion **MUST NOT** be joined in a single section.
- Short Communications and Case Reports should be divided into appropriate sections.
- Products, equipments and materials: the trade name must be followed by the manufacturer's name, city, state and country, within parentheses upon first mention. For further mentions, only the manufacturer's name is required.

- All abbreviations must be explained at first mention.

**Title page**

- The first page must contain the title of the manuscript, a short title (maximum of 40 characters, to be used as a running head), author(s) name(s) (no more than 6) and their Department(s), School(s) and/or University (s). **DO NOT INCLUDE** the author's titles (DDS, MSc, PhD, etc.) or position (Professor, Graduate student, etc.).
- Provide the name and **complete** address of the corresponding author (inform email, telephone and fax numbers).

**Summary**

- The second page should contain a summary of no more than 250 words, stating the aims, methods, results, and any conclusions drawn from the study. Do not use topics and paragraphs and do not cite references in the Summary.
- A list of key words (no more than 5) should be included below the summary in lowercase letters, separated by commas.

**Introduction**

- Summarize the purpose of the study, giving only pertinent references. Do not review existing literature extensively. State clearly the working hypothesis.

**Material and Methods**

- Material and methods should be presented in sufficient detail to allow confirmation of the observations. **Indicate the statistical methods used, if applicable.**

**Results**

- Present the results in a logical sequence in the text, tables and figures, emphasizing the important information.
- Do not repeat in the text data contained in the tables and illustrations. The important observations should be emphasized.
- Do not repeat the same data in tables and figures.
- Describe the statistical data in this section.

**Discussion**

- Summarize the findings without repeating in detail the data given in the Results section.
- Relate your observations to other relevant studies and point out the implications of the findings and their limitations. Cite pertinent studies.
- Present your conclusions at the end of the Discussion, indicating how your study is pertinent and/or its clinical implications. Presentation of the conclusions in topics should be avoided.

**Summary in Portuguese (for Brazilian authors only)**

- The Summary in Portuguese should be **IDENTICAL** to the English version (Summary). **DO NOT INCLUDE** title and key words in Portuguese.

**Acknowledgements**

- Financial support by government agencies should be acknowledged. If appropriate, technical assistance or assistance from colleagues may be acknowledged.

**References**

- References must follow the Journal's style. Authors should refer to a current issue of the BDJ for guidance on reference citation and presentation of the reference list.
- References must be numbered consecutively in the text in order of citation, within parentheses, without space between numbers: (1), (3,5,8), (10-15). **DO NOT USE** superscript numbers.
- For papers with two authors, cite both authors in the text, as follows: Ex: "According to Santos **and** Silva (1)...". If there are more than 3 authors, cite only the first author and add "et al.". Ex: "Pécora et al. (2) reported that..."
- All authors of each paper should be included in the Reference List unless there are 7 or more. In this case, the first 6 authors should be given, followed by "et al.".
- The reference list must be typed at the end of the manuscript in numerical sequence. **No more than 25 references may be cited.**
- Citation of abstracts and books, as well as articles published in non-indexed journals should be avoided, unless absolutely necessary. **Do not cite references in Portuguese.**

- Abbreviations of journal titles should conform to those used in Dental Index. The style and punctuation of references must follow the format illustrated below:

*Journal articles*

1. Lea SC, Landini G, Walmsley AD. A novel method for the evaluation of powered toothbrush oscillation characteristics. Am J Dent 2004;17:307-309.

*Book*

2. Shafer WG, Hine MK, Levy BM. A Textbook of Oral Pathology. 4th ed. Philadelphia: WB Saunders; 1983.

*Chapter in a Book*

3. Walton RE, Rotstein I. Bleaching discolored teeth: internal and external. In: Principles and Practice of Endodontics. Walton RE (Editor). 2nd ed. Philadelphia: WB Saunders; 1996. p 385-400.

**Tables**

- Each table with its title must be typed after the text. Tables should be numbered with Arabic numerals. **DO NOT USE** vertical lines, bold letters and capital letters (except the initials).
- The corresponding title should appear at the top of each table.
- Tables must contain all necessary information and be understandable without allusions to the text.

**Figures**

- **BDJ WILL NOT ACCEPT FIGURES EMBEDDED IN FILES ORIGINATED IN TEXT-EDITING SOFTWARE (WORD OR SIMILAR) OR FIGURES ORIGINATED IN POWER POINT.**
- The digital files of the images should be generated in Photoshop, Corel or any other image-editing software and saved in the CD-ROM. Image files should have TIFF extension and 300 dpi minimum resolution. Only BLACK & WHITE figures are accepted. Save the figures in the CD-ROM.
- Lettering and identifying marks must be clear and sharp, and the critical areas of x-rays and photomicrographs must be demarcated and/or isolated.
- Separate parts of composite figures must be labeled with capital letters (A, B, C, etc). Single figures and composite figures must have minimum width of 8 cm and 16 cm,

respectively.

- Figure captions should be numbered with Arabic numerals and typed on a separate page, after the lists of references or after the tables (if any)

Submission of manuscripts

**CHECKLIST FOR AUTHORS PRIOR TO SUBMISSION**

1. Submission letter;
2. 1 (one) printed copy of the manuscript (including tables and figure captions) along with 1 (one) printed copy of each figure (if any) on good quality plain paper.
3. CD-Rom containing:

- the manuscript file in Word (text, tables, figure captions and figures).
- the digital files of figures, black & white, saved in TIFF format with minimum resolution of 300 dpi.

4. In the manuscript, observe:

- identification of authors only on the title page.
- text typed in Times New Roman 12 font, with 1.5 spacing, 2.5-cm margins at each side.
- tables, figure captions and figures at the end of the manuscript.

ANEXO D: Normas de Publicação American Journal of Orthodontics and Dentofacial Orthopedics

**Information for Authors**

**Electronic manuscript submission and review**

The *American Journal of Orthodontics and Dentofacial Orthopedics* uses the *Elsevier Editorial System (EES)*, an online manuscript submission and review system.
To submit or review an article, please go to the **AJO-DO** EES website: ees.elsevier.com/ajodo .

Send other correspondence to:

Dr. Vincent G. Kokich, DDS, MSD, Editor-in-Chief
*American Journal of Orthodontics and Dentofacial Orthopedics*
University of Washington
Department of Orthodontics, D-569
HSC Box 357446
Seattle, WA 98195-7446
Telephone (206) 221-5413
E-mail: vgkokich@u.washington.edu

**General Information**

The *American Journal of Orthodontics and Dentofacial Orthopedics* publishes original research, reviews, case reports, clinical material, and other material related to orthodontics and dentofacial orthopedics.

Submitted manuscripts must be original, written in English, and not published or under consideration elsewhere. Manuscripts will be reviewed by the editor and consultants and are subject to editorial revision. Authors should follow the guidelines below.

Statements and opinions expressed in the articles and communications herein are those of the author(s) and not necessarily those of the editor(s) or publisher, and the editor( s) and publisher disclaim any responsibility or liability for such material. Neither the editor(s) nor the publisher guarantees, warrants, or endorses any product or service advertised in this publication; neither do they guarantee any claimmade by themanufacturer of any product or service. Each reader must determine whether to act on the informationin this publication, and neither the *Journal* nor its sponsoring organizations shall be liable for any injury due to the publication of erroneous information.

**Guidelines for Original Articles**

Submit Original Articles via EES: ees.elsevier.com/ajodo .

Before you begin, please review the guidelines below. To view a 7-minute video explaining how to prepare your article for submission, go to Video on Manuscript Preparation.

1. Title Page. Put all information pertaining to the authors in a separate document. Include the title of the article, full name(s) of the author(s), academic degrees, and institutional affiliations and positions; identify the corresponding author and include an address, telephone and fax numbers, and an e-mail address. This information will not be available to the reviewers.
2. Abstract. Structured abstracts of 200 words or less are preferred. A structured abstract contains the following sections: Introduction, describing the problem; Methods, describing how the study was performed; Results, describing the primary results; and Conclusions, reporting what the authors conclude from the findings and any clinical implications.
3. Manuscript. The manuscript proper should be organized in the following sections: Introduction and literature review, Material and Methods, Results, Discussion, Conclusions, References, and figure captions. Express measurements in metric units, whenever practical. Refer to teeth by their full name or their FDI tooth number. For style questions, refer to the *AMA Manual of Style*, 9th edition. Cite references selectively, and number them in the order cited. Make sure that all references have been mentioned in the text. Follow the format for references in "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Ann Intern Med 1997;126:36-47); http://www.icmje.org . Include the list of references with the manuscript proper. Submit figures and tables separately (see below); do not embed figures in the word processing document.

4. Figures. Digital images should be in TIF or EPS format, CMYK or grayscale, at least 5 inches wide and at least 300 pixels per inch (118 pixels per cm).Do not embed images in a word processing program. If published, images could be reduced to 1 column width (about 3 inches), so authors should ensure that figures will remain legible at that scale. For best results, avoid screening, shading, and colored backgrounds; use the simplest patterns available to indicate differences in charts. If a figure has been previously published, the legend (included in the manuscript proper) must give full credit to the original source, and written permisson from the original publisher must be included. Be sure you have mentioned each figure, in order, in the text.

5. Tables. Tables should be self-explanatory and should supplement, not duplicate, the text. Number them with Roman numerals, in the order they are mentioned in the text. Provide a brief title for each. If a table has been previously published, include a footnote in the table giving full credit to the original source and include written permission for its use from the copyright holder. Submit tables as text-based files (Word or Excel, for example) and not as graphic elements.

6. Model release and permission forms. Photographs of identifiable persons must be accompanied by a release signed by the person or both living parents or the guardian of minors. Illustrations or tables that have appeared in copyrighted material must be accompanied by written permission for their use from the copyright owner and original author, and the legend must properly credit the source. Permission also must be obtained to use modified tables or figures.

7. Copyright release. In accordance with the Copyright Act of 1976, which became effective February 1, 1978, all manuscripts must be accompanied by the following written statement, signed by all authors:

*"The undersigned author(s) transfers all copyright ownership of the manuscript* ***[insert title of article here]*** *to the American Association of Orthodontists in the event the work is published. The undersigned author(s) warrants that the article is original, does not infringe upon any copyright or other proprietary right of any third party, is not under consideration by another journal, has not been previously published, and includes any product that may derive from the published journal, whether print or electronic media. I (we) sign for and accept responsibility for releasing this material."* Scan the printed copyright release and submit it via EES.

8. Use the International College of Medical Journal Editors Form for the Disclosure of Conflict of Interest (ICMJE Conflict of Interest Form). If the manuscript is accepted, the disclosed information will be published with the article. The usual and customary listing of sources of support and institutional affiliations on the title page is proper and does not imply a conflict of

interest. Guest editorials, Letters, and Review articles may be rejected if a conflict of interest exists.

9. Institutional Review Board approval. For those articles that report on the results of experiments of treatments where patients or animals have been used as the sample, Institutional Review Board (IRB) approval is mandatory. No experimental studies will be sent out for review without an IRB approval accompanying the manuscript submission.

10. Systematic Reviews and Meta-Analyses must be accompanied by the current PRISMA checklist and flow diagram (go to Video on CONSORT and PRISMA). For complete instructions, see our Guidelines for Systematic Reviews and Meta-Analyses.

11. Randomized Clinical Trials must be accompanied by the current CONSORT statement, checklist, and flow diagram (go to Video on CONSORT and PRISMA). For complete instructions, see our Guidelines for Randomized Clinical Trials.

**Other Articles**

Follow the guidelines above, with the following exceptions, and submit via EES.

Case Reports will be evaluated for completeness and quality of records, quality of treatment, uniqueness of the case, and quality of the manuscript. A high quality manuscript must include the following sections: introduction; diagnosis; etiology; treatment objectives, treatment alternatives, treatment progress, and treatment results; and discussion. The submitted figures must include extraoral and intraoral photographs and dental casts, panoramic radiographs, cephalometric radiographs, and tracings from both pretreatment and posttreatment, and progress or retention figures as appropriate. Complete Case Report Guidelines can be downloaded from Case Report Guidelines

Techno Bytes items report on emerging technological developments and products for use by orthodontists.

**Miscellaneous Submissions**

Letters to the Editor and their responses appear in the Readers' Forum section and are encouraged to stimulate healthy discourse between authors and our readers. Letters to the Editor must refer to an article that was published within the previous six (6) months and must be less than 500 words including references. Send letters or questions directly to the editor, via e-mail:

vgkokich@u.washington.edu. Submit a signed copyright release with the letter.

Brief, substantiated commentary on subjects of interest to the orthodontic profession is published occasionally as a Special Article. Submit Guest Editorials and Special Articles via the Web site.

Books and monographs (domestic and foreign) will be reviewed, depending upon their interest and value to subscribers. Send books to the Editor in Chief, Dr. Vincent G. Kokich, Department of Orthodontics, University of Washington D-569, HSC Box 357446, Seattle,WA98195-7446. They will not be returned.

**Checklist for authors**

____Title page, including full name, academic degrees, and institutional affiliation and position of each author, and author to whom correspondence and reprint requests are to be sent, including address, business and home phone numbers, fax numbers, and e-mail address
____Abstract
____Article proper, including references and figure legends
____Figures, in TIF or EPS format
____Tables
____Copyright release statement, signed by all authors
____Photographic consent statement(s)
____ICMJE Conflict of interest statement
____Permissions to reproduce previously published material

ANEXO E: Artigos Publicados

**Pazzini CA, Pereira LJ, Carlos RG, de Melo GE, Zampini MA, Marques LS. Nickel: periodontal status and blood parameters in allergic orthodontic patients. Am J Orthod Dentofacial Orthop. 2011 Jan;139(1):55-9.**


**Introduction**

The aims of this study were to compare blood parameters and periodontal characteristics of orthodontic patients allergic to nickel with those of nonallergic patients and to determine the correlation between blood components and periodontal abnormalities.

**Methods**

Ninety-six randomly selected patients participated in the study. After determining the prevalence of those allergic to nickel, 2 groups were formed: 16 allergic patients and 16 nonallergic patients. Allergies to nickel were diagnosed by using the patch test, periodontal conditions were determined by using the gingival index, and humoral characteristics were determined through a complete blood test, including the quantification of nickel in the blood and the immunoglobulin E level. Feces examinations were performed to control for parasitic infections. Periodontal evaluations were performed blindly. Statistical analysis included the unpaired t test, the Mann-Whitney test, and the Pearson and Spearman correlations ($P \leq 0.05$).

**Results**

Statistically significant differences between groups were found for bands ($P = 0.001$). There was no correlation between the concentration of nickel and immunoglobulin E level ($P = 0.674$, experimental group; $P = 0.605$, control group). However, there was a positive correlation between the gingival index and the number of bands ($P = 0.05$).

**Conclusions**

Nickel can have an influence over the periodontal status of allergic orthodontic patients, causing an increase in band quantification that was correlated to gingival index.


Allergic and inflammatory reactions have commonly been associated with release of metal ions, especially nickel, during orthodontic treatment.[1] Although corrosion of orthodontic devices occurs, it does not appear to result in significant destruction of metallic components or have significant detrimental effects on mechanical properties.[2] However, several studies have established an association between nickel and allergic manifestations in orthodontic patients. [3], [4], [5], [6] and [7]

A critical analysis of the literature showed a lack of consistent evidence on the subject.[8] Part of this problem is due to the methodologic limitations in most studies (study design, sample size and characterization, type of evaluation), leading to conflicting results and compromising clinical decision making.

The prevalence of nickel sensitization is near 10% in the general population, and its frequency in women is about 5 times higher than in men.[9] Nickel has often been pointed out as a biologic sensitizer capable of causing short-term and long-term sensitivity reactions: type IV immune response, cell-mediated by T-lymphocytes.[10] However, during orthodontic treatment, sensitive patients are at greater risk of oral discomfort, which hinders both hygiene and treatment.[11] More than a direct sensitizing agent of skin and mucosa, nickel appears to alter periodontal status, acting as a modifying factor of periodontal disease in sensitive patients and creating inflammatory patterns of immune reactions.

In such a context, a number of questions should be raised. What are the clinical and biologic implications of an allergy to nickel for orthodontic patients? Are the effects associated with the buildup of nickel

throughout orthodontic treatment or the local release of the ions? Are reactions mediated by an allergic or inflammatory response?

The aim of this study was to determine the correlation between blood parameters and periodontal abnormalities in orthodontic patients allergic and nonallergic to nickel.

**Material and methods**

Ninety-six patients awaiting orthodontic treatment at a specialization course in orthodontics of the Vale do Rio Verde University, Três Corações, Brazil, were randomly selected for participation in the study. All were white; 58 (60%) were female, and 32 (40%) were male; their ages ranged from 10 to 43 years.

All subjects began orthodontic treatment at the same time (in January 2006). Before the placement of the appliances, all participants received prophylaxis with bicarbonate spray and orientation on oral hygiene. Morelli braces (Sorocaba, São Paulo, Brazil) were attached; these braces have the following composition: 16% to 18% chrome, 10% to 14% nickel, and 2% to 3% molybdenum.

Regarding the periodontal aspects, clinical gingival characteristics such as color and volume were assessed. By using a standardized millimeter probe, the presence or absence of gingival bleeding on probing in the region of the maxillary and mandibular first premolars was assessed at 3 points on the vestibular, lingual, mesial, and distal faces. The Loe[12] index was used for periodontal status assessment, with the following classification: 0, normal gingiva; 1, mild inflammation, slight change in color, with no bleeding on probing; 2, moderate inflammation, reddish appearance, mild edema, bleeding on probing; and 3, severe inflammation, reddish appearance, clear edema, ulceration, tendency toward spontaneous bleeding. The Loe index was based on mean values of 4 first premolars multiplied by 3 sites per tooth. However, the mean value is used to make a score, which classifies the patient into 4 categories. First premolars were selected because of their location at the halfway point of each quadrant of the oral cavity. In general, first molars are preferred for periodontal status evaluation. However, they had bands, and periodontal status could not be evaluated in those teeth. The Loe index takes into account qualitative changes in the gingival tissue. If a patient had at least 2 of the classifications of each previous item, he or she was placed in the more severe category.

Assessments of periodontal status were carried out by 1 blinded, duly calibrated (kappa >0.90) examiner at regular 3-month intervals for 12 months (4 evaluations: T1, T2, T3, and T4) with braces in place. Additionally, prophylaxis with bicarbonate spray was performed at each session (after the periodontal evaluation).

The skin patch test was used for the diagnosis of nickel allergy. This is the most efficient method for confirming the etiologic diagnosis of allergic-contact eczema and consists of a 2 × 2 cm patch (Finn Chambers) attached to the dorsal region of the patient at 2 points 10 cm apart, after cleansing of the skin with cotton soaked in alcohol. Because of the extensive area involved, an ideal amount of the gel (standardized by the manufacturer) containing a 5% nickel sulfate antigen (solid petroleum jelly) (Epitest Ltd Oy, Tuusula, Finland) remained for 48 hours. During the placement of the patches, patients were instructed to remove them if they experienced any reaction beyond the expected and to call the researchers in charge and go to the municipal medical emergency room. After 48 hours, the patches were removed, and only 1 reading was made in compliance with the norms of the International Contact Dermatitis Research Group.[13]

Its guidelines are as follows: (–) negative; (+) discrete erythema with some papules; (++) erythema, papules and vesicles; (+++) intense erythema, papules, and vesicles. All patients considered negative had no clinical condition visible to the naked eye, and all patients considered positive had erythema, edema, papules, and blisters (+++).

Nine months after the beginning of treatment, the prevalence of patients with a nickel allergy was determined by using the patch test. At this time, 1 subject with intraoral piercing, 1 who abandoned treatment for personal reasons, and 1 who was pregnant were excluded from the study. Sixteen patients

(17.2%) were determined to have a nickel allergy and formed the allergic group. Among the nonallergic subjects, 16 were randomly selected to form the age-paired control group. After the prevalence of those allergic to nickel was determined and the control group was formed, all 32 participants underwent a full blood test, assessment of total immunoglobin E (IgE), and determination of the amount of circulating nickel in the blood throughout the orthodontic treatment. For the examinations, 6 mL of blood was vacuum collected (vacuo-time system) from all patients after they fasted for 8 hours. For the blood count, 3 mL of blood in a vacuum tube with the EDTA anticoagulant was analyzed on an automated hematology analyzer, by using the ABX Micros CRP device (OT-CT-OS-CS, France). The differential count of blood cells was performed through a blood smear without anticoagulant, stained with Single Prov stain (NewProv - eosin methylene blue solution of cyclohexadiene to 0.1%), viewed under a microscope and read, with the aim of immersion and platelet count. For the evaluation of total IgE, 1 mL of serum without anticoagulant was analyzed by using the chemoluminescence method on the Inmulite equipment. Two 2 mL of blood was used to assess the amount of circulating nickel, collected in a trace tube for atomic absorption spectrophotometry (graphite kiln with Zeeman corrector). In the blood count, analyses were performed of leukocytes, basophils, eosinophils, myelocytes, metamyelocytes, bands, neutrophils, lymphocytes, and monocytes.

Feces examinations were performed on all 32 participants to determine parasitic infestations (helminth eggs and larvae, protazoon cysts) that might affect the white blood cell count, especially the number of eosinophils. The feces were collected in a sterile container, and patients were instructed to avoid contamination, using the method of Hoffman, Pons, and Janer,[14] with centrifugation and spontaneous sedimentation of recent feces with no conservatives. The material was placed on a slide and assessed under a microscope (Alphaphot, 2Y52, Nikon, New York, NY). All laboratory examinations were performed by a duly trained pharmacist-biochemist (M.A.Z.). Patients who had verminosis (1 from the allergic group and 2 from the nonallergic group) were excluded from the statistical analysis of the blood components because the parasitosis could impact the results of the blood test.

**Statistical analysis**

Statistical analysis included the unpaired t test and the Mann-Whitney test for the intergroup comparison of the blood components, Pearson and Spearman coefficients for correlating the amount of circulating nickel with the IgE (immunoglobin of the allergy), and the Loe index at T3 with the number of bands. Differences were considered significant at $P \leq 0.05$.

The study received approval from the Research Ethics Committee of the Vale do Rio Verde University, Três Corações, Brazil, under process number 0001.0.380.000-07.

**Results**

Preliminary analyses were conducted between the groups. The results in Table I refer to the blood parameters evaluated in both groups. Only data comprising white cells are presented, since red cells are not related to immune response. The number of bands was the only variable with a statistically significant difference between groups, with higher values found in allergic patients.

Subsequently, a correlation test was conducted between circulating nickel and IgE quantification in the groups. There was no significant correlation for those variables, demonstrating that the amount of nickel in the blood did not influence the specific antibody production (Table II).

Additionally, Table III shows the correlation between the gingival index (T3) and the number of bands in the allergic and nonallergic groups. The gingival index was used every 3 months for a total period of 12 months. However, only T3 was used for blood correlations, since it coincided with the blood-sample collection. Interestingly, the number of bands was significantly related to the gingival index, demonstrating that periodontal status influenced the number of young neutrophils.

## Discussion

In this study, we aimed to evaluate the relationship between periodontal status and blood parameters in allergic and nonallergic patients. To find real allergic patients and to prevent iatrogenic sensitization, a questionnaire was used to investigate previous symptoms of contact dermatitis.[15] However, self-reported information on metal dermatitis as an estimate of nickel allergy has low validity.[9] Thus, the patch test was applied 9 months after treatment started. The patch skin test is the most efficient method for confirming the etiologic diagnosis of contact allergy.[16] The prevalence of nickel allergy in this study was reported previously[17] and corroborates results of other authors. [18], [19], [20] and [21]

Nickel is known to potentially cause allergic reactions in the oral cavity. [11] and [22] However, we found no correlation between the circulating nickel levels and IgE quantification for either allergic or nonallergic patients (Table II). Although nickel is a strong sensitizer in the development of contact allergy, adverse reactions related to orthodontic appliances are rare.[23] Few allergic reactions seem to be associated with the use of allergens in dental materials because the oral mucosal membranes are less reactive than the skin.[24] Another factor that could explain the absence of allergic reactions in the oral cavity is the development of tolerance to nickel.[20]

One patient in the allergic group with generalized gingival hyperplasia during orthodontic treatment had a biopsy in the maxillary anterior region between the central incisors that was sent for histopathologic analysis. The results of the biopsy showed that the fragment had stratified hyperplasic squamous epithelium, with islands of conjunctive tissue. In the underlying conjunctive tissue, there were chronic inflammatory cells (especially plasmocytes) and newly formed blood vessels, suggesting gingival hyperplasia (inflammatory response).

The number of bands significantly increased in allergic patients when compared with nonallergic subjects. Bands are stimulated in bacterial infections or inflammations. In this case, the increase of bands in allergic patients might be explained by the difference in the gingival index at the same time. It was suggested that nickel can influence local and systemic inflammatory reactions throughout orthodontic treatment. The warm, moist, aerobic status of the mouth offers a favorable environment for the activity of microorganisms. Since orthodontic appliances hamper oral hygiene, dental biofilm accumulates with greater facility on tooth surfaces and the appliances in most patients. Consequently, periodontal disease can cause neutrophils to increase. [25] and [26] Nickel seems to adhere to endogenous macromolecules, thereby stimulating the proliferation of monocytes, macrophages, and cytotoxic cells; these might influence the periodontal inflammatory response. [27], [28] and [29] Furthermore, nickel induces T lymphocytes to produce cytokines, including interferon IF-λ and interleukin IL-2, IL-5, and IL-10, stimulating tissue proliferation, which could favor gingival hyperplasia.[30]

Orthodontic treatment with braces containing nickel does not appear to have a direct allergenic effect on the gingival tissues.[31] However, the results of our study indicate that nickel can influence the condition of the periodontal and blood cells of allergic orthodontic patients, but with reactions of an inflammatory, rather than allergic, nature.

Although these results do not establish an association between allergic reactions and nickel in orthodontic accessories, orthodontists should be aware of the medical history of their patients and seek alternatives, such as braces with a low nickel content, as well as strictly monitoring patients' dental hygiene.[32] Further studies addressing chrome or cobalt (also present in orthodontic accessories) levels in the blood are needed, thereby allowing a better understanding of the exact nature of the immunologic systemic reactions involved in the inflammatory process in allergic patients.

## Conclusions

Our results indicate that nickel could have an influence on the periodontal status of allergic orthodontic patients, causing an increase in band quantification that is correlated to the gingival index.

**References**

1. Barrett RD, Bishara SE, Quinn JK. Biodegradation of orthodontic appliances. Part I. Biodegradation of nickel and chromium in vitro. Am J Orthod Dentofacial Orthop 1993;103:8-14.
2. House K, Sernetz F, Dymock D, Sandy JR, Ireland AJ. Corrosion of orthodontic appliances—should we care? Am J Orthod Dentofacial Orthop 2008;133:584-92.
3. Fors R, Persson M. Nickel in dental plaque and saliva in patients with and without orthodontic appliances. Eur J Orthod 2006;28:292-7.
4. Levrini L, Lusvardi G, Gentile D. Nickel ions release in patients with fixed orthodontic appliances. Minerva Stomatol 2006;55:115-21.
5. Minang JT, Arestr€om I, Zuber B, J€onsson G, Trove-Blomberg M, Ahlborg N. Nickel-induced IL-10 down-regulates Th1- but not Th2-type cytokine responses to the contact allergen nickel. Clin Exp Immunol 2006;143:494-502.
6. Setcos JC,Babaei-MahaniA, Silvio LD,Mj€or IA,WilsonNH. The safety of nickel containing dental alloys. Dent Mater 2006;22:1163-8.
7. Kao CT, Ding SJ, He H, Chou MY, Huang TH. The cytotoxicity of orthodontic metal bracket immersion media. Eur J Orthod 2007;29:198-203.
8. Kolokitha OE, Kaklamanos EG, Papadopoulos MA. Prevalence of nickel hypersensitivity in orthodontic patients: a meta-analysis. Am J Orthod Dentofacial Orthop 2008;134:722.e1-12; discussion, 722-3.
9. Fors R, Persson M, Bergstr€om E, Stenlund H, Stymne B, Stenberg B. Nickel allergy—prevalence in a population of Swedish youths from patch test and questionnaire data. Contact Dermatitis 2008;58:80-7.
10. Schmalz G, Garhammer P. Biological interactions of dental alloys with oral tissues. Dent Mater 2002;18:396-406.
11. Marigo M, Nouer DF, Genelhu MC, Malaquias LC, Pizziolo VR, Costa AS, et al. Evaluation of immunologic profile in patients with nickel sensitivity due to use of fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 2003;124:46-52.
12. Loe H. The gingival index, the plaque index and the retention index systems. J Periodontol 1967;38(Suppl):610-6.
13. Rietschel R, Fowler JF, editors. Fisher's contact dermatitis. 4th ed. Baltimore: Willians & Wilkins; 1995.
14. J_unior ESS, Garcia-Zapata MTA. Laboratory diagnosis of opportunistic intestinal parasites with emphasis on human microsporidiosis, in Goiania-Go. Rev Soc Bras Med Trop 2006;39:560-4.
15. Ramadan AA. Effect of nickel and chromium on gingival tissues during orthodontic treatment: a longitudinal study. World J Orthod 2004;5:230-5.
16. James WD, Rosenthal LE, Brancaccio RR, Marks JG Jr. American Academy of Dermatology patch testing survey: use and effectiveness of this procedure. J Am Acad Dermatol 1992;26:991-4.
17. Pazzini CA, Oliveira G Jr, Marques LS, Pereira CV, Pereira LJ. Prevalence of nickel allergy and longitudinal evaluation of periodontal abnormalities in orthodontic allergic patients. Angle Orthod 2009; 79:922-7.
18. Peltonen L. Nickel sensitivity in the general population. Contact Dermatitis 1979;5:27-32.
19. Greig DG. Contact dermatitis reaction to a metal buckle on a cervical headgear. Br Dent J 1983;155:61-2.
20. Kerosuo H, Moe G, Hensten-Pettersen A. Salivary nickel and chromium in subjects with different types of fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 1997;111:595-8.
21. Janson GR, Dainesi EA, Consolaro A, Woodside DG, de Freitas MR. Nickel hypersensitivity reaction before, during, and after orthodontic therapy. Am J Orthod Dentofacial Orthop 1998;113: 655-60.
22. Kerosuo H, Kullaa A, Kerosuo E, Kanerva L, Hensten-Pettersen A. Nickel allergy in adolescents in relation to orthodontic treatment and piercing of ears. Am J Orthod Dentofacial Orthop 1996;109: 148-54.
23. Schuster G, Reichle R, Bauer RR, Schopf PM. Allergies induced by orthodontic alloys: incidence and impact on treatment. Results of a survey in private orthodontic offices in the Federal State of Hesse, Germany. J Orofac Orthop 2004;65:48-59.
24. Haberman A, Pratt M, Storrs T. Contact dermatitis from beryllium in dental alloys. Contact Dermatitis 1993;28:157-62.
25. Ujiie Y, Oida S, Gomi K, Arai T, Fukae M. Neutrophil elastase is involved in the initial destruction of human periodontal ligament. J Periodontal Res 2007;42:325-30.

26. Petropoulos G, McKay IJ, Hughes FJ. The association between neutrophil numbers and interleukin-1 alpha concentrations in gingival crevicular fluid of smokers and non-smokers with periodontal disease. J Clin Periodontol 2004;31:390-5.
27. Wataha IC, Sun ZL, Hanks CT, Fang DN. Effect of Ni ions on expression of intercellular adhesion molecule 1 by endothelial cells. J Biomed Mater Res 1997;36:145-51.
28. Wataha JC, Ratanasathien S, Hanks CT, Sun Z. In vitro IL-l beta and TNF-alpha release from THP-1 monocytes in response to metal ions. Dent Mater 1996;12:322-7.
29. Wataha JC, Lockwood PE, Marek M, Ghazi M. Ability of Nicontaining biomedical alloys to activate monocytes and endothelial cells vitro. J Biomed Mater Res 1999;45:251-7.
30. Fernandez-Botran R, Sanders VM, Mosmann TR, Vitetta ES. Lymphokine-mediated regulation of the proliferative response of clones of T helper 1 and T helper 2 cells. J Exp Med 1988;168:543-58.
31. Saglam AM, Baysal V, Ceylan AM. Nickel and cobalt hypersensitive reaction before and after orthodontic therapy in children. J Comtemp Dent Pract 2004;5:79-90.
32. Pantuzo MC, Zen_obio EG, de Andrade Marigo H, Zen_obio MA. Hypersensitivity to conventional and nickel-free orthodontic brackets. Braz Oral Res 2007;21:298-302.

**Pazzini CA, Marques LS, Ramos-Jorge ML, Júnior GO, Pereira LJ, Paiva SM. Longitudinal assessment of periodontal status in patients with nickel allergy treated with conventional and nickel-free braces. Angle Orthod. 2012 Jul;82(4):653-7.**


**Abstract**

**Objective:** To perform a longitudinal comparison of periodontal status in allergic individuals treated with conventional and nickel-free braces.

**Materials and Methods:** Forty-two individuals allergic to nickel were randomly divided into two groups: those receiving conventional braces (n = 21) and those receiving nickel-free braces (n = 21). Periodontal status (gingival hyperplasia, change in color and bleeding) was assessed before treatment (T0) and at 3-month intervals for 12 months (T1, T2, T3, and T4), using the Löe Index. Evaluations were performed blindly by a single, calibrated examiner, followed by prophylaxis and orientations regarding oral hygiene. Data were analyzed using the Mann-Whitney *U*-test for comparisons of the gingival index between groups and Friedman's test for successive comparisons between sessions in the same group ($P \leq .05$).

**Results:** Periodontal status did not differ between groups in the initial 9 months of treatment, whereas significant differences were found at T3 and T4 (.039 and .047, respectively). Individuals wearing conventional appliances had higher mean gingival index scores than those wearing nickel-free braces.

**Conclusion:** Individuals with an allergy to nickel exhibit better periodontal health when treated with nickel-free braces than with conventional braces.


Keywords: Nickel allergy, Orthodontic treatment, Nickel-free braces

## INTRODUCTION

Studies indicate nickel as a cause of alterations in periodontal status among allergic orthodontic patients.1–6 At the same time, there has been a tendency on the part of the industry to produce orthodontic materials with low concentrations of nickel (0.2% to 4%). Such appliances are denominated nickel-free and are advertised as releasing low quantities of nickel ions, which may diminish the allergic response in sensitive patients.7–10

A critical evaluation of the literature reveals little consistent evidence regarding the actual effectiveness of nickel-free appliances in orthodontic patients allergic to nickel. In a recent systematic review, Pazzini et al.4 found only four studies suggesting that nickel-free braces may favor orthodontic treatment in allergic patients, and the authors stress the need for clinical trials that can provide more consistent evidence with regard to this issue. Studies involving nickel-free braces may provide important information by first determining whether nickel is truly the agent responsible for triggering responses of an inflammatory and/or allergic nature. Moreover, such studies would determine whether nickel-free braces actually represent a viable alternative for patients who are allergic to nickel.

The aim of the present study was to perform a longitudinal comparison of periodontal status in allergic individuals treated with conventional and nickel-free braces.

## MATERIALS AND METHODS

Eighty patients awaiting treatment at an orthodontic specialization course of the Centro Universitário de Lavras (Lavras, MG, Brazil) were randomly selected for participation in the study. All participants were white; 47 (58%) were female and 33 (42%) were male, ranging in age from 10 to 45 years.

All individuals began treatment in January 2009. Before placement of the appliances, all participants received prophylaxis with bicarbonate spray and orientations regarding oral hygiene (the oral hygiene measures consisted of brushing the teeth at least four times a day and using dental floss with the help of

needle and mouthwash rinse to facilitate the removal of plaque). Morelli brackets (Dental Morelli, Sorocaba, SP, Brazil) were used. The conventional appliances contained 16% to 20% chrome, 8% to 13% nickel, and 2% to 3% molybdenum; the nickel-free appliances contained up to 18% chrome, 0.2% to 4% nickel, and 3.5% molybdenum.

After treatment was begun, a skin patch test was performed to identify patients with nickel allergy. According to the allergy evaluation standards of the Brazilian Medical Association and the Federal Medicine Council (Brazilian Study Group on Contact Dermatitis, 2000), this is the most efficient method of confirming the etiologic diagnosis of allergic contact eczema. The method requires use of a 2 × 2-cm patch (Finn Chambers, Tuusula, Finland) attached to the dorsal region of the patient at two different points placed 10 cm apart, following cleansing of the skin with cotton soaked in alcohol. Owing to the extensive area involved, an ideal amount of the gel (standardized by the manufacturer) containing a 5% nickel sulfate antigen (solid petroleum jelly) (FDA Allergenic, Rio de Janeiro, Brazil, Importer and Distributor; Epitest Ltd Oy, Tuusula, Finland) remained in place for 48 hours. Patients were instructed to remove the patches if they experienced any reaction beyond the expected, and to contact the researchers in charge, as well as the municipal medical emergency room. After 48 hours, the patches were removed, and a single reading was performed in compliance with the norms of the International Contact Dermatitis Research Group11: (-) negative; (+) discrete erythema with some papules; (++) erythema, papules, and vesicles; and (+++) intense erythema, papules, and vesicles. All patients considered negative presented no clinical condition visible to the naked eye, and all patients considered positive presented erythema, edema, papules, and blisters (+++). At the time, seven individuals abandoned treatment for personal reasons. Forty-two individuals (57.5%) proved allergic to nickel and were randomly distributed into two groups: those receiving conventional braces (group I; n = 21), and those receiving nickel-free braces (group II; n = 21).

Clinical gingival characteristics (color, volume, and bleeding) were assessed. A standardized probe with a millimeter ruler was used to determine the presence or absence of gingival bleeding around the upper and lower first premolars at three different points on the vestibular, palatine/lingual, mesial, and distal faces. These teeth were selected owing to their location at the halfway point of each quadrant of the oral cavity. For the evaluation, the Löe Gingival Index12,13 was used, with qualitative changes in the gingival tissue taken into consideration. The Löe Index is based on mean scores of the first premolars, multiplied by three sites per tooth. The mean value is used to classify the patient into one of four categories, with the following scores: 0—normal gums; 1—mild inflammation, slight change in color, mild edema, no bleeding upon probing; 2—moderate inflammation, reddish appearance, mild edema, bleeding upon probing; and 3—severe inflammation, reddish appearance, evident edema, ulceration, and tendency toward spontaneous bleeding. Patients with at least two of the classifications of each previous item were classified in the more severe category.

Assessments of periodontal status were carried out by a single, blinded, duly calibrated examiner (kappa >0.90) before that start of treatment ($T_0$) and at regular 3-month intervals for 12 months (total of five evaluations: $T_0$, $T_1$, $T_2$, $T_3$, $T_4$) with braces in place. Additionally, prophylaxis with a bicarbonate spray was performed in each session (following the periodontal evaluation).

Intergroup (conventional and nickel-free) and intragroup comparisons of the gingival index in the five evaluation sessions were performed using chi-square and Friedman's tests, respectively. Nonparametric tests were applied because the Löe Index is a qualitative method. Thus, values for the 0, 1, 2, and 3 scores cannot be used as ordinary variables, and they were dichotomized as presence (1, 2, or 3) and absence (0) of gingival inflammation for statistical purposes. Differences were considered significant with $P \leq .05$. This study received approval from the Human Research Ethics Committee of the Centro Universitário de Lavras (Brazil) under process number 0015.0189.000-10.

## RESULTS

A 57.5% prevalence of nickel allergy (42 individuals) was noted, 67% (28) of which occurred in female patients and 33% (14) in male patients. The study started with 21 patients in each group. However, only 20 from the conventional group and 17 from the nickel-free groups completed all treatment sessions. Table 1 and Figure 1 display median Löe Index values and percentiles (25th, 75th) for the groups with regard to periodontal status. Because the data did not present a normal distribution, median values were

presented instead of means, and interquartile distribution was presented (25% and 75%) instead of standard deviation. Significant differences between groups were detected at $T_3$ and $T_4$ ($P \leq .05$). Table 2 displays intragroup findings, revealing significant differences among different evaluation times ($P \leq .05$).

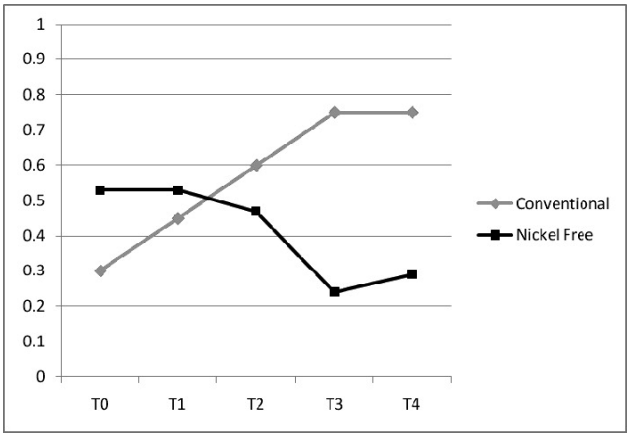



Figure 1: Mean gingival index scores at five evaluation times ($T_0$, $T_1$, $T_2$, $T_3$, $T_4$).

| Time | Group | Median | 25th, 75th Interquartiles | *P* Value |
|---|---|---|---|---|
| $T_0$ | Conventional | 0 | 0.00, 1.00 | .286 |
| | Nickel-free | 0 | 0.00, 1.00 | |
| $T_1$ | Conventional | 0 | 0.00, 1.00 | .457 |
| | Nickel-free | 0 | 0.00, 1.00 | |
| $T_2$ | Conventional | 0.5 | 0.00, 1.00 | .368 |
| | Nickel-free | 0 | 0.00, 1.00 | |
| $T_3$ | Conventional | 1 | 0.00, 1.00 | **.026** |
| | Nickel-free | 0.23 | 0.00, 0.50 | |
| $T_4$ | Conventional | 1 | 0.00, 1.00 | **.031** |
| | Nickel-free | 0 | 0.00, 1.00 | |

Table1: Gingival Index at Five Evaluation Times ($T_0$, $T_1$, $T_2$, $T_3$, $T_4$) (Chi-Square Test)

| | Conventional (n = 20) | Nickel-Free (n = 17) |
|---|---|---|
| $T_0$ | 0.30[a] | 0.53[a] |
| $T_1$ | 0.45[a] | 0.53[a] |
| $T_2$ | 0.60[a,b] | 0.47[a] |
| $T_3$ | 0.75[b] | 0.23[a] |
| $T_4$ | 0.75[b] | 0.29[a] |

[a,b] Different superscript letters indicate significant differences between lines within the same column.

Table 2: Comparison of Mean Gingival Index Scores Over Time in Conventional and Nickel-Free Groups (Friedman's Test)

**DISCUSSION**

Patients treated with conventional braces exhibited greater periodontal alterations than those treated with nickel-free braces. Scores between the two groups did not differ at $T_0$, $T_1$, and $T_2$, which suggests that both groups had the same periodontal status at baseline and over the first 9 months of treatment. Differences occurred only on the $T_3$ and $T_4$ evaluations (9 and 12 months after beginning treatment, respectively), suggesting a cumulative effect of nickel throughout orthodontic treatment. This finding is in agreement with previous studies.2,3,14,15 However, other authors16,17 have reported different results, which may be explained by the limited follow-up period (1 to 5 months), as well as the considerable variety in diagnostic method, study design, sample size, and research approach.

The prevalence of nickel allergy in the present study was 57.5%, and most allergic individuals were female (2 : 1 ratio). This is consistent with findings described by other authors.17,18 Greater sensitivity to nickel on the part of women is related to environmental exposure, such as contact with detergents, jewelry, and other metallic objects, whereas such sensitivity in men is related to professional exposure, especially among those who handle nickel.

Nickel is widely used in the manufacture of orthodontic appliances.19 However, few studies have addressed the influence of this metal on periodontal health, especially in a longitudinal fashion. Despite the fact that gingival inflammation is considered an allergic reaction to metals in orthodontic appliances,20 the onset of periodontal disease depends mainly on the accumulation of biofilm. Placement of orthodontic braces influences the accumulation of biofilm and the colonization of bacteria, thereby leading to a greater proneness to inflammation and bleeding.21 This indicates that inflammatory conditions of the disease may be transitory, stemming from variations in the degree of oral hygiene. In the present study, biofilm may have contributed to camouflaging the periodontal status of individuals. However, this is not believed to have been a source of bias, because all individuals received the same instructions with regard to oral hygiene before and throughout orthodontic treatment.

Nickel is more than a direct sensitizing agent of skin and mucosa; it appears to alter periodontal status, acting as a modifying factor of periodontal disease in sensitive patients. The increase in the Löe Index over time ($T_3$ and $T_4$) in the conventional group and the decrease in the nickel-free group suggest nickel adhesion to endogenous macromolecules, stimulating the proliferation of monocytes, macrophages, and cytotoxic cells, which may affect the periodontal inflammatory response.22,23

Furthermore, nickel induces T lymphocytes to produce cytokines, including interferon (IF)-γ, as well as interleukins (IL)-2, -5, and -10, thereby stimulating tissue proliferation, which may favor gingival hyperplasia.24 Therefore, it would be plausible to presuppose that the continuous release of small amounts of nickel to the epithelium could be an initiating factor of gingival overgrowth induced by orthodontic therapy.25

The type and duration of oral exposure to nickel alloys capable of initiating an adverse reaction remain controversial issues. Metal ions in the saliva can be swallowed before they cause a reaction or may be

absorbed in the mouth, and the amount of nickel released from dental alloys is significantly lower than that consumed as part of food ingestion.26,27 In the present study, periodontal abnormalities differed between groups only after 9 months, demonstrating that the reaction is dependent on exposure time.2,3 Release of nickel from orthodontic appliances has been demonstrated in a number of in vitro studies.28 The release of 40 mg of nickel per day occurs with appliances spanning the entire mouth. However, daily consumption of nickel in the diet ranges from 300 mg to 600 mg, which suggests a predominantly local rather than systemic effect of nickel. In some patients with a positive patch test to nickel sulfate, perpetuation of recurrent aphthous stomatitis may be related to hypersensitivity to ingested nickel salts, independently of local contact with nickel.29

Nickel-free braces have been evaluated with regard to chemical composition and behavioral characteristics.30,31 Nickel-free stainless steel braces produced by Morelli (Monobloc) do not have a significant amount of nickel in their composition and may be a viable alternative for allergic patients, in agreement with previous studies.4,9 However, this composition decreases their mechanical properties, because nickel considerably enhances resistance to oxidation and corrosion.32 Thus, the presented results are relative specifically to the Morelli brackets.

Although the present study carried out a longitudinal evaluation of periodontal status in individuals allergic to nickel, the immunologic aspects involved were not considered. Thus, additional studies are needed, to address humoral aspects and favor a better understanding of the mechanisms involved.

## CONCLUSIONS

Based on findings of the present study:

- Individuals with an allergy to nickel exhibit better periodontal health when treated with nickel-free braces than with conventional braces.

**Acknowledgments**

This study was supported by CAPES.

## REFERENCES

1. Marigo, M. , D. F. Nouer , M. C. Genelhu , L. C. Malaquias , V. R. Pizziolo , A. S. Costa , O. A. Martins-Filho , and L. F. Alves-Oliveira . Evaluation of immunologic profile in patients with nickel sensitivity due to use of fixed orthodontic appliances. Am J Orthod Dentofacial Orthop 2003. 124:46–52. [CrossRef]
2. Pazzini, C. A. , G. O. Júnior , L. S. Marques , C. V. Pereira , and L. J. Pereira . Prevalence of nickel allergy and longitudinal evaluation of periodontal abnormalities in orthodontic allergic patients. Angle Orthod 2009. 79:922–927. [Abstract][Medline]
3. Pazzini, C. A. , L. J. Pereira , L. S. Marques , R. Generoso , and G. de Oliveira Jr . Allergy to nickel in orthodontic patients: clinical and histopathologic evaluation. Gen Dent 2010. 58:58–61.
4. Pazzini, C. A. , L. S. Marques , L. J. Pereira , P. Corrêa-Faria , and S. M. Paiva . Allergic reactions and nickel-free braces: a systematic review. Braz Oral Res 2011. 25:85–90. [CrossRef]
5. Johansson, K. , H. Kerosuo , and K. Lammintausta . Nickel sensitization in orthodontically treated and non-treated female adolescents. Contact Dermatitis 2011. 64:132–137. [CrossRef]
6. Garcia-Gavin, J. , J. C. Armario-Hito , V. Fernández-Rondo , J. M. Fernández-Vozmediano , J. Sánchez-Pérez , J. F. Silvestre , W. Uter , and A. M. Giménez-Arnau . Nickel allergy in Spain needs active intervention. Contact Dermatitis 2011. 64:289–291. [CrossRef]
7. Mockers, O. , D. Deroze , and J. Camps . Cytotoxicity of orthodontic bands, brackets and archwires in

vitro. Dent Mater 2002. 18:311–317. [CrossRef]

8. Rahilly, G. and N. Price . Nickel allergy and orthodontics. J Orthod 2003. 30:171–174. [CrossRef]
9. Pantuzo, M. C. G. , E. G. Zenóbio , H. A. Marigo , and M. A. F. Zenóbio . Hypersensitivity to conventional and to nickel-free orthodontic brackets. Braz Oral Res 2007. 21:298–302. [CrossRef]
10. Pazzini, C. A. , L. J. Pereira , R. G. Carlos , G. E. de Melo , M. A. Zampini , and L. S. Marques . Nickel: periodontal status and blood parameters in allergic orthodontic patients. Am J Orthod Dentofacial Orthop 2011. 139:55–59. [CrossRef]
11. Nonaka, H. , T. Nakada , M. Iijima , and H. I. Maibach . Metal patch test results from 1990–2009. J Dermatol 2011. 38:267–271. [CrossRef]
12. Loe, H. and J. Silness . Periodontal disease in pregnancy. Acta Odontol Scand 1963. 21:533–551. [CrossRef] [Medline]
13. Loe, H. The gingival index, the plaque index and retention index systems. J Periodontol 1967. 38:610–616. [CrossRef] [Medline]
14. Agaoglu, G. , T. Arun , B. Izgi , A. Yarat , and B. Izgu . Nickel and chromium levels in the saliva and serum of patients with fixed orthodontic appliances. Angle Orthod 2001. 71:375–379. [Abstract]
15. Petoumenou, E. , M. Arndt , L. Keilig , S. Reimann , H. Hoederath , T. Eliades , A. Jäger , and C. Bourauel . Nickel concentration in the saliva of patients with nickel-titanium orthodontic appliances. Am J Orthod Dentofacial Orthop 2009. 135:59–65. [CrossRef]
16. Bishara, S. E. , R. D. Barrett , and M. I. Selim . Biodegradation of orthodontic appliances. Part II. Changes in the blood level of nickel. Am J Orthod Dentofacial Orthop 1993. 103:115–119. [CrossRef]
17. Singh, D. P. , V. Sehgal , K. L. Pradhan , A. Chandna , and R. Gupta . Estimation of nickel and chromium in saliva of patients with fixed orthodontic appliances. World J Orthod 2008. 9:196–202.
18. Kolokitha, O. E. , E. G. Kaklamanos , and M. A. Papadopoulos . Prevalence of nickel hypersensitivity in orthodontic patients: a meta-analysis. Am J Orthod Dentofacial Orthop 2008. 134:722–734. [CrossRef]
19. Saglam, A. M. S. , V. Baysal , and A. M. Ceylan . Nickel and cobalt hypersensitivity reaction before and after orthodontic therapy in children. J Contemp Dent Pract 2004. 4:79–90.
20. Ramadan, A. A. Effect of nickel and chromium on gingival tissues during orthodontic treatment: a longitudinal study. World J Orthod 2004. 5:230–234.
21. Naranjo, A. A. , M. L. Triviño , A. Jaramillo , M. Betancourth , and J. E. Botero . Changes in the subgingival microbiota and periodontal parameters before and 3 months after bracket placement. Am J Orthod Dentofacial Orthop 2006. 130:17–22. [CrossRef]
22. Wataha, I. C. , Z. L. Sun , C. T. Hanks , and D. N. Fang . Effect of Ni ions on expression of intercellular adhesion molecule 1 by endothelial cells. J Biomed Mater Res 1997. 36:145–151. [CrossRef]
23. Wataha, J. C. , P. E. Lockwood , M. Marek , and M. Ghazi . Ability of Ni containing biomedical alloys to activate monocytes and endothelial cells in vitro. J Biomed Mater Res 1999. 45:251–257. [CrossRef]
24. Faria, A. M. and H. L. Weiner . Oral tolerance: therapeutic implications for autoimmune diseases. Clin Dev Immunol 2006. 13:143–157. [CrossRef]
25. Gursoy, U. K. , O. Sokucu , V. J. Uitto , A. Aydin , S. Demirer , H. Toker , O. Erdem , and A. Sayal . The role of nickel accumulation and epithelial cell proliferation in orthodontic treatment induced overgrowth. Eur J Orthod 2007. 29:555–558. [CrossRef]
26. Kerosuo, H. , G. Moe , and E. Kleven . In vitro release of nickel and chromium from different types of simulated orthodontic appliances. Angle Orthod 1995. 65:111–116. [Abstract][Medline]
27. Setcos, J. C. , A. Babaei-Mahani , L. D. Silvio , I. A. Mjör , and N. H. Wilson . The safety of nickel containing dental alloys. Dent Mater 2006. 22:1163–1168. [CrossRef]
28. Eliades, T. and A. E. Athanasiou . In vivo aging of orthodontic alloys: implications for corrosion potential, nickel release, and biocompatibility. Angle Orthod 2002. 72:222–237. [Abstract][Medline]
29. Pacor, M. L. , G. Di Lorenzo , N. Martinelli , et al. Results of double-blind placebo controlled challenge with nickel salts in patients affected by recurrent aphthous stomatitis. Int Arch Allergy

Immunol 2003. 131:296–300. [CrossRef]

30. Assad-Loss, T. F. , R. M. L. Neves , and J. N. Mucha . Elemental composition and superficial aspect of metallic brackets slot. Rev Dent Press Ortod Ortop Facial 2008. 13:85–96. [CrossRef]

31. Haddad, A. C. , A. Tortamano , A. L. Souza , and P. V. Oliveira . An *in vitro* comparison of nickel and chromium release from brackets. Braz Oral Res 2009. 23:399–406. [CrossRef]

32. Bakhtari, A. , T. G. Bradley , W. K. Lobb , and D. W. Berzins . Galvanic corrosion between various combinations of orthodontic brackets and archwires. Am J Orthod Dentofacial Orthop 2011. 140:25–31. [CrossRef]

# PRODUÇÃO CIENTÍFICA

## PRODUÇÃO CIENTÍFICA

### Apresentação de trabalhos em eventos científicos

- Soares MF, Campos ACV, Pazzini CA, Fonseca DC, Paiva SM, Ferreira EF. Empowerment in Brazilian: Access and use of public oral health. IADR, 2012.
- Pazzini CA, Pereira LJ, Peconick AP, Paiva SM, Marques LS. Níquel: Avaliação periodontal e sanguínea em pacientes ortodônticos alérgicos após a remoção do aparelho. Brazilian Oral Research 2011; 25(Suppl):7-446.

⇨ Trabalho apresentado na 28ª Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica realizada na cidade de Águas de Lindóia, SP, no período de 3 a 6 de Setembro de 2011.

- Martins-Junior PA, Pazzini CA, Marques LS, Pereira LJ, Silva TA, Paiva SM. Alterações nos níveis de citocinas inflamatórias de pacientes alérgicos ao níquel tratados com braquetes convencional e níquel free. Brazilian Oral Research 2011;25(Suppl):7-446.

⇨ Trabalho apresentado na 28ª Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica realizada na cidade de Águas de Lindóia, SP, no período de 3 a 6 de Setembro de 2011.

- Pazzini CA, Marques LS, Pereira LJ, Paiva SM. Efetividade de braquetes níquel free: uma revisão sistemática. Brazilian Oral Research 2010;24(Suppl):3-456.

⇨ Trabalho apresentado na 27ª Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica realizada na cidade de Águas de Lindóia, SP, no período de 9 a 12 de Setembro de 2010.

- Pazzini CA, Marques LS, Pereira LJ, Paiva SM. Estudo longitudinal das alterações sanguíneas e periodontais em pacientes alérgicos ao níquel sob tratamento ortodôntico. Encontro do grupo de Professores de Ortodontia e Odontopediatria. Apresentação oral.

⇨ Trabalho apresentado no 41° Encontro do Grupo Brasileiro de Professores de Ortodontia e Odontopediatria realizado na cidade de Campos do Jordão, SP, no período de 7 a 9 de Junho de 2010.

- Pazzini CA, Generoso RC, Pereira LJ, Marques LS. Avaliação periodontal e sanguínea de pacientes ortodônticos alérgicos ao níquel: Estudo Coorte Prospectivo. Brazilian Oral Research 2010;23(Suppl):1-408.

⇨ Trabalho apresentado na 26ª Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica realizada na cidade de Águas de Lindóia, SP, no período de 9 a 13 de Setembro de 2009.

**Resumos de trabalhos publicados em anais de eventos científicos**

- Pazzini CA, Pereira LJ, Peconick AP, Paiva SM, Marques LS. Níquel: Avaliação periodontal e sanguínea em pacientes ortodônticos alérgicos após a remoção do aparelho. Brazilian Oral Research 2011;25(Suppl):7-446.

⇨ Trabalho apresentado na 28ª Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica realizada na cidade de Águas de Lindóia, SP, no período de 3 a 6 de Setembro de 2011.

- Martins-Junior PA, Pazzini CA, Marques LS, Pereira LJ, Silva TA, Paiva SM. Alterações nos níveis de citocinas inflamatórias de pacientes alérgicos ao níquel

tratados com braquetes convencional e níquel free. Brazilian Oral Research 2011;25(Suppl):7-446.

⇨ Trabalho apresentado na 28ª Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica realizada na cidade de Águas de Lindóia, SP, no período de 3 a 6 de Setembro de 2011.

- Pazzini CA, Marques LS, Pereira LJ, Paiva SM. Efetividade de braquetes níquel free: uma revisão sistemática. Brazilian Oral Research 2010;24(Suppl):3-456.

⇨ Trabalho apresentado na 27ª Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica realizada na cidade de Águas de Lindóia, SP, no período de 9 a 12 de Setembro de 2010.

- Pazzini CA, Generoso RC, Pereira LJ, Marques LS. Avaliação Periodontal e sanguínea de pacientes ortodônticos alérgicos ao níquel: Estudo Coorte Prospectivo. Brazilian Oral Research 2010;23(Suppl):1-408.

⇨ Trabalho apresentado na 26ª Reunião Anual da Sociedade Brasileira de Pesquisa Odontológica realizada na cidade de Águas de Lindóia, SP, no período de 9 a 13 de Setembro de 2009.

Artigos científicos publicados

- Pazzini CA, Marques LS, Ramos-jorge M L, Júnior, GO, Pereira LJ, Paiva SM. Longitudinal assessment of periodontal status in patients with nickel allergy treated with conventional and nickel-free braces. Angle Orthod. 2012;82:653-57

- Marques LS, Pazzini CA, Pantuzo MCG. Nickel: Humoral and periodontal changes in orthodontic patients. Dental Press J Orthod. 2012 Mar-Apr;17(2):15-17.
- Pazzini CA, Marques LS, Pereira LJ, Corrêa-Faria P, Paiva SM. Allergic reactions and nickel-free braces: A systematic review. Braz Oral Res. 2011; 25:85-90.
- Armond MC, Generoso RC, Pazzini CA, Pereira LJ, Marques LS. Crohn'sDisease: clinical manifestations of orthodontic interest. Am J Orthod Dentofacial Orthop. 2011; 139:704-7.
- Pazzini CA, Pereira LJ, Generoso RC, Brito-melo GEA, Zampini MA, Marques LS. Nickel: periodontal status and blood parameters in allergic orthodontic. Am J Orthod Dentofacial Orthop 2011; 139:55-9.
- Pazzini CA, Pereira LJ, Marques LS, Generoso RC, Júnior, GO. Allergy to nickel in Orthodontic patients: Clinical and histopathologic evaluation. Gen Dent. 2010;58:58-61.
- Marques LS, Generoso RC, Armond MC, Pazzini CA. Short-root anomaly in an orthodontic patient. Am J Orthod Dentofacial Orthop. 2010;138:346-8.
- Pazzini CA, Júnior GO, Marques LS, Pereira CV, Pereira LJ. Prevalence of Nickel Allergy and Longitudinal Evaluation of Periodontal Abnormalities in Orthodontic Allergic Patients. Angle Orthod. 2009;79:922-7.

Artigos científicos submetidos á publicação

Pazzini CA, Pereira LJ, Peconick AP, Marques LS, Paiva SM. Periodontal status and blood parameters in orthodontic patients with nickel allergy following braces removal. Brazilian Dental Journal, 2012.

# RESUMO

**Condições sanguíneas e gengivais em pacientes alérgicos ao níquel submetidos a tratamento ortodôntico com braquetes convencionais e níquel free**


## RESUMO

Este estudo teve como objetivo identificar em uma população de pacientes ortodônticos aqueles que são alérgicos ao níquel, bem como verificar se existem diferenças entre esses indivíduos, considerando especificamente a condição gengival e sanguínea em caráter longitudinal, através da utilização de braquetes convencionais e níquel free no tratamento ortodôntico. A população do estudo foi composta por 80 pacientes, de ambos os gêneros, faixa etária 10 anos e 8 meses a 43 anos e 1 mês. Para diagnóstico da alergia ao níquel, foram realizados testes de contato 1 mês antes do início do tratamento. Quarenta e dois pacientes apresentaram alergia e foram divididos de forma randomizada em 2 grupos: Grupo I (n=21), tratados com braquetes convencionais e Grupo II (n=21), tratados com braquetes níquel free. Todos os indivíduos participantes realizaram Hemograma completo, avaliação da Imunoglobulina E total e quantificação de níquel circulante, antes do tratamento ortodôntico ($T_0$) e durante o tratamento ortodôntico (9 meses após o início- $T_3$). Os aspectos gengivais foram avaliados pelo índice gengival de Löe antes do tratamento e durante o tratamento (com intervalo de 3 meses entre as avaliações) totalizando 5 avaliações ($T_0$: antes do início do tratamento; $T_1$: 3 meses após o início do tratamento; $T_2$: 6 meses após o início do tratamento; $T_3$: 9 meses após o início do tratamento; $T_4$: 12 meses após o início do tratamento). Os dados foram analisados utilizando o teste Mann Whitney, Anova de Friedman, T pareado e não pareado, Wilcoxon e Correlação de Pearson e Spearman ($p \leq .05$). A condição gengival não diferiu entre os grupos nos 6 meses iniciais de tratamento, enquanto foram encontradas diferenças significativas em $T_3$ e $T_4$ (0.026 e 0.031 – Anexo E). Não houve diferenças

significativas no sangue entre os grupos antes do início do tratamento ($p>.05$). Durante o tratamento ortodôntico ($T_3$), o número de segmentados diminui no grupo níquel free. Houve um aumento do número de basófilos e concentração de níquel de $T_0$ para $T_3$ ($p\leq.05$) em ambos os grupos. O número de eosinófilos e os níveis de IgE diminuíram significativamente ($p\leq.05$). Não houve correlação significativa entre a concentração de níquel e índice gengival, IgE, basófilos, eosinófilos, bastões e segmentados ($p>.05$). Indivíduos tratados com braquetes níquel free apresentaram menores desordens sanguíneas e alterações gengivais do que indivíduos tratados com braquetes convencionais, porém, ainda dentro da normalidade.

**Descritores:** alergia, níquel, tratamento ortodôntico, braquetes níquel free

# ABSTRACT

**Conditions blood and gingival in nickel allergic patients undergoing orthodontic treatment with conventional and nickel free braces**


## ABSTRACT

This study aimed to identify in a population of orthodontics patients who are allergic to nickel, as well as verifying the differences between these individuals, specifically considering the gingival status and blood in a longitudinal study through the use of conventional and nickel free braces in orthodontic treatment. The study population was represented by 80 patients of both genders, aged 10 years and 8 months to 43 years and 1 month. For diagnosis of nickel allergy, contact tests were performed in these individuals. After the testing of contact allergy, 42 patients were divided randomly into 2 groups: Group I (n = 21) treated with conventional brace and Group II (n = 21) treated with nickel free braces. All subjects underwent complete blood count, total immunoglobulin E assessment and quantification of the amount of nickel present in the circulating blood, before orthodontic treatment ($T_0$), during treatment (approximately 9 months after the start-$T_3$) and one month after removal apparatus ($T_5$). Gingival aspects were evaluated by Gingival Index of Löe before treatment, during treatment (at 3 months interval between assessments) and 1 month after removal of the devices, totaling six ratings ($T_0$: prior to initiation of treatment; $T_1$: 3 months after initiation of treatment, $T_2$: 6 months after initiation of treatment, $T_3$: 9 months after initiation of treatment, $T_4$: 12 months after initiation of treatment; $T_5$: 1 month after removing the handset), for a single calibrated examiner and blindly. Data were analyzed using the Mann-Whitney, Friedman, T-paired and unpaired, Wilcoxon and Spearman and Pearson correlation ($p \leq .05$). Periodontal status did not differ between the groups in the initial 9

months of treatment, while significant differences were found in $T_3$ and $T_4$ (0.026 and 0.031). No significant differences in the blood between groups before the initiation of treatment ($p>.05$). During orthodontic treatment ($T_3$), the number of targeted decreases in the nickel free group. There was an increase in the number of basophils and nickel concentration from $T_0$ to $T_3$ ($p\leq.05$) for both groups. However, the number of eosinophils and IgE levels decreased significantly ($p\leq.05$). There was no significant correlation between the concentration of nickel and gingival index, level of IgE, basophils, eosinophils, and segmented rods ($p>.05$). Individuals with an allergy to nickel have better periodontal health and humoral when treated with nickel free braces.

**Key words:** allergy, nickel, orthodontic treatment, nickel free braces

## LISTA DE ABREVIATURAS E SIGLAS

| | |
|---|---|
| **µg** | Microgramas |
| **Ni-Ti** | Níquel Titânio |
| **mL** | Mililitros |
| **ICP-AES** | Espectroscopia de Emissão de Plasma-Atômico Acoplado Indutivamente |
| **DNA** | Ácido desoxirribonucleico |
| **FDA** | *Food and Drug Administration* |
| **%** | Percentual |
| **IgA** | Imunoglobulina A |
| **Cm** | Centímetro |
| **®** | Marca registrada |
| **IgE** | Imunoglobulina E |
| **ICDRG** | *International Contact Dermatitis Research Group* |
| **GBEDC** | Grupo Brasileiro de estudos em dermatite de contato |
| **UFMG** | Universidade Federal de Minas Gerais |
| **UNILAVRAS** | Centro Universitário de Lavras |
| **COEP** | Comitê de Ética em Pesquisa com Seres Humanos |
| **OMS** | Organização Mundial da Saúde |
| **p** | *p-value* |
| **PROCAD** | Programa Nacional de Cooperação Acadêmica |
| **WHO** | *World Health Organization* |
| **IL** | Interleucina |
| **EDTA** | Ácido etilenodiamino tetra-acético |
| **$T_H$** | Células T Helper |
| **$CD4^+$** | Grupamento de diferenciação 4 |
| **NFAT** | Fator nuclear de células T ativadas |
| **INF-γ** | Interferon |
| **IG** | Índice Gengival |
| **GI** | *Gingival Index* |

## LISTA DE FIGURAS

## LISTA DE QUADROS

## LISTA DE TABELAS

### ARTIGO 1



### ARTIGO 2

**SUMÁRIO**

# CONSIDERAÇÕES INICIAIS

## CONSIDERAÇÕES INICIAIS

A maioria dos dispositivos ortodônticos como bandas, braquetes, fios e arcos são confeccionados a partir do aço inoxidável austenítico, que contém cerca de 8 a 20% de níquel em sua composição (Gursoy et al., 2005; Sória et al., 2005). O níquel, por sua vez, tem sido frequentemente apontado como sensibilizador biológico, capaz de induzir reações de hipersensibilidade tardia (Peltonen, 1979; Silvennoinen-kassinen et al., 1992; Santucci et al., 1993; Janson et al., 1998). Conhecida como resposta imune Tipo IV, apresenta sinais não específicos na cavidade bucal como hiperplasia gengival, queilite angular, ardência na boca, gosto metálico, descamação labial e periodontite (Marigo et al., 2003). Reações alérgicas e inflamatórias aos metais são comuns e o níquel é considerado o maior causador delas (Bordji et al., 1996; Huang et al., 2004; Saito et al., 2011). Pelo fato dos fios ortodônticos e braquetes manterem proximidade com a mucosa oral por períodos longos de tempo, estes precisam ser resistentes a corrosão e liberação de íons e não devem gerar respostas alérgicas, devendo ser bem tolerados pelos tecidos bucais (Evans e Durning, 1996). Entretanto, a sensibilização ao níquel pode se desenvolver durante o tratamento ortodôntico (Johansson et al., 2011).

A grande procura pelo tratamento ortodôntico fez com que as reações adversas decorrentes da utilização de aparelhos ortodônticos fixos e removíveis se tornassem motivo de preocupação tanto para os ortodontistas quanto para pesquisadores da área de saúde (Fors e Persson, 2006; Levrini et al., 2006; Minang et al., 2006; Setcos et al., 2006; Kao et al., 2007; Pereira et al., 2008, Pazzini et al., 2009, 2010).

Os primeiros relatos de alergia ao níquel datam de 1979, quando foi estimado que 4,5% a 28,5% da população apresentaram hipersensibilidade ao níquel, com a maior

prevalência nas mulheres, suspeitando-se que a maior sensibilização seja deflagrada pela utilização de bijuterias (Peltonen, 1979; Janson et al., 1998).

A liberação de níquel de aparelhos ortodônticos foi observada em vários estudos "in vitro". A liberação de íons das ligas dentárias existe, porém, acredita-se na falta de toxicidade destas ligas, pois de acordo com alguns estudos, a liberação é menor que a quantidade ingerida através da dieta (Park e Shearer, 1983; Jones et al., 1986; Barrett et al., 1993; Bishara et al., 1993).

Embora estudos recentes tenham abordado a influência do níquel no desenvolvimento de reações alérgicas em pacientes ortodônticos (Marigo et al., 2003; Fischer et al., 2007; Spiewak et al., 2007; Pantuzo et al., 2007; Pazzini et al., 2009, Saito et al., 2011), desvendar este mecanismo representa um desafio tanto para os ortodontistas quanto para pesquisadores da área de saúde. Entretanto, a análise crítica da literatura revela que, em sua maior parte, as evidências fornecidas pelas pesquisas são ainda pouco consistentes. Parte desse problema ocorre em virtude de limitações metodológicas (desenho dos estudos, tamanho e natureza da amostra e tipo de avaliação diagnóstica), conduzindo a resultados conflitantes, que podem comprometer a tomada de decisões clínicas. Além disso, existem questionamentos que esses trabalhos não respondem, tais como:

- Quais as implicações de natureza clínica e biológica da alergia ao níquel?

- Os efeitos estão associados ao acúmulo de níquel ao longo do tratamento ortodôntico ou à liberação local?

- As reações são mediadas por uma resposta imunológica sistêmica e/ou modificação da resposta inflamatória local no periodonto?

- Qual a melhor conduta para o ortodontista clínico frente ao paciente alérgico ao níquel?

Industrialmente já se observa a tendência de fabricação dos acessórios ortodônticos com baixa concentração de níquel (de 0,2 a 4,0%), ainda que se saiba da consequente piora nas suas propriedades mecânicas, pois o níquel tem a finalidade de melhorar consideravelmente a resistência à corrosão e à oxidação, desfavorecendo a mecânica ortodôntica (Platt et al., 1997). Esses dispositivos são conhecidos como níquel free, por ser um material capaz de liberar baixas quantidades de íons níquel, o que pode diminuir a sensibilidade em pacientes alérgicos, tornando-se uma boa opção para pacientes hipersensíveis a este metal (Hamula et al., 1996; Rahilly e Price, 2003; Menezes et al., 2004; Pantuzo et al., 2007).

Portanto, o objetivo deste trabalho foi identificar em uma população de pacientes ortodônticos, aqueles que são alérgicos ao níquel, bem como verificar se existem diferenças entre esses indivíduos, considerando especificamente a condição gengival e sanguínea em caráter longitudinal, através da utilização de braquetes convencionais e braquetes níquel free no tratamento ortodôntico. Este trabalho foi desenvolvido junto ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Minas Gerais.

# MATERIAL E MÉTODOS

## MATERIAL E MÉTODOS

Este trabalho teve início após a aprovação do Comitê de Ética em Pesquisa em Seres Humanos da Universidade Federal de Minas Gerais (COEP/UFMG) com protocolo 0125.0203.203-11 e do Centro Universitário de Lavras (COEP/UNILAVRAS) com protocolo 0015.0189.000-10 (Anexos A e B). Durante a realização das pesquisas foram seguidos todos os princípios éticos necessários.

Todos os voluntários somente foram convidados a participar da pesquisa após esclarecimentos sobre os riscos e benefícios da mesma e após a assinatura de um Termo de Consentimento Livre e Esclarecido detalhadamente explicado (Apêndices A, B e C). Os pacientes não tiveram nenhum custo para a participação no estudo.

### Constituição da amostra

A população do estudo foi representada por 80 pacientes, de ambos os gêneros, sendo 47 pacientes do gênero feminino (58%) e 33 pacientes do gênero masculino (42%), com início do tratamento ortodôntico agendado na clínica do Curso de Pós-Graduação em Ortodontia do Centro Universitário de Lavras- UNILAVRAS.

Para diagnóstico da alergia ao níquel foram realizados testes de contato nesses 80 indivíduos, orientado por um médico alergista. De acordo com a Associação Médica Brasileira e o Conselho Federal de Medicina (GBEDC, 2000), este é o método mais eficiente para confirmar o diagnóstico etiológico do eczema alérgico de contato. Consiste de um emplasto (*FINN CHAMBERS*® - FDA allergenic, Scanpor® Tuusula, Finland) de 2 cm x 2 cm fixado na região dorsal do paciente em dois pontos distintos

com distância de 10 cm entre eles. Os emplastos foram fixados após a limpeza da pele com algodão embebido em álcool. Por se tratar de uma área extensa, foi colocada uma quantidade do gel, padronizada pelo fabricante, através de um medidor, por 48 horas, contendo o antígeno sulfato de níquel (vaselina sólida) a 5% (*FDA allergenic*, Patchkit standard new generation®, Tuusula, Finland) (Figuras 1- A e B e Figura 2). Durante a instalação dos emplastos, os pacientes foram orientados a removê-los caso observassem alguma reação diferente do esperado e a procurarem os pesquisadores responsáveis e o pronto-atendimento médico municipal. Após as 48 horas, estes testes foram removidos e a leitura realizada de acordo com as normas preconizadas pelo International Contact Dermatitis Research Group – ICDRG (Rietschel, Fowler, 1995; GBEDC, 2000): (-) negativo; (+) discreto eritema com algumas pápulas; (++) eritema, pápulas e vesículas; (+++) intenso eritema, pápulas e vesículas confluentes (Figura 3).

Dos 80 pacientes iniciais, somente 73 pacientes aceitaram participar do estudo. Após a realização dos testes de contato, 42 pacientes apresentaram alergia ao níquel (57,5%) e 2 grupos foram formados, de forma randomizada, sendo que, todos os indivíduos eram alérgicos ao níquel. No Grupo I (n=21), os pacientes foram tratados com braquetes Convencionais e no Grupo II (n=21), os pacientes foram tratados com braquetes Níquel free. A faixa etária dos indivíduos alérgicos variou de 10 anos e 8 meses a 43 anos e 1 mês.

Antes da montagem dos aparelhos, os pacientes receberam profilaxia prévia com jato de bicarbonato, além de orientações sobre higiene bucal. Foram utilizados braquetes da marca Morelli ® (Sorocaba/SP/Brasil) Convencionais (Prescrição Roth) e Níquel free (Prescrição Roth Monobloc), slot .022” x .030” (0,56 x 0,76 mm).

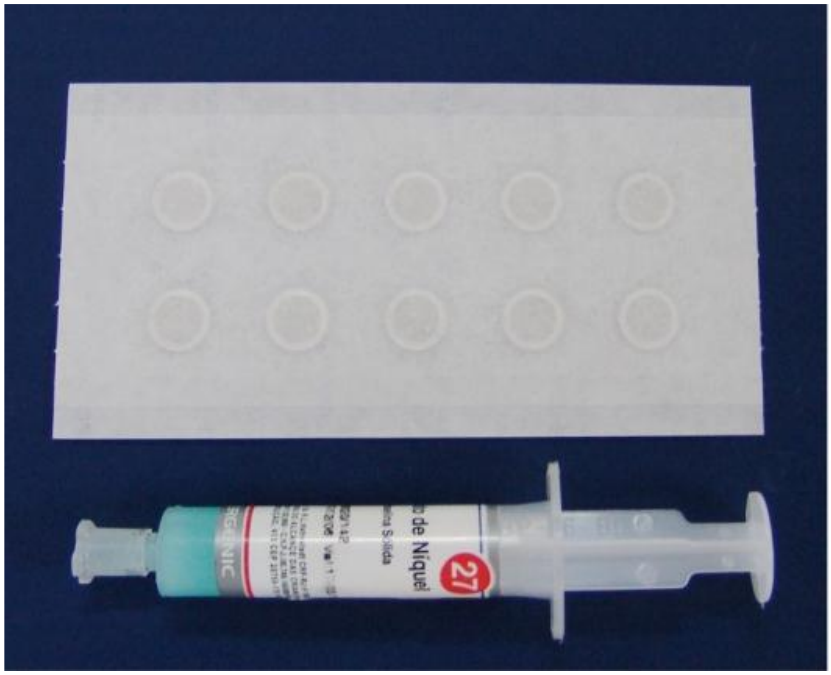



Figura 1A: Kit do teste de contato *(FDA allergenic)*

Figura 1B: *Finn chambers* com o antígeno sulfato de níquel

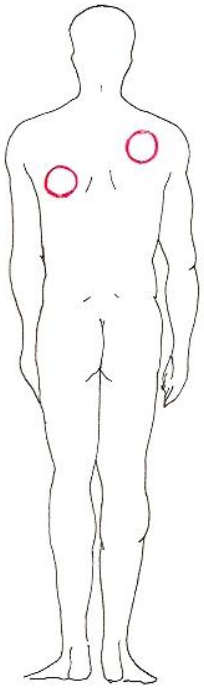


Figura 2: Esquema demonstrando o local de implantação do teste de contato

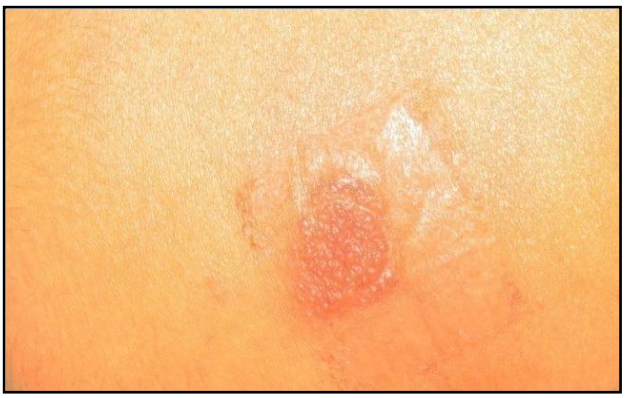


Figura 3: Reação positiva ao teste de contato grau +++

**Exames Complementares**

*Avaliação sanguínea*

Todos os indivíduos participantes realizaram Hemograma completo, avaliação da Imunoglobulina E total (IgE total) e quantificação do níquel circulante presente no sangue, antes do tratamento ortodôntico ($T_0$) e durante o tratamento ortodôntico (9 meses após o início do tratamento- $T_3$).

Para a realização dos exames, realizou-se a coleta de 6 ml de sangue a vácuo (sistema vácuo-time) com todos os pacientes em jejum de oito horas. Para o hemograma, utilizou-se 3 ml de sangue em tubo a vácuo com anticoagulante EDTA, por um sistema automatizado *(Automated Hematology Analyzer)* pelo aparelho ABX Micros CRP (modelo OT-CT-OS-CS, França). Realizou-se a contagem diferencial de células sanguíneas mediante confecção de esfregaço sanguíneo sem anticoagulante e corada com o corante Single Prov *(NewProv)*, levados ao microscópio e feita a leitura com objetiva de imersão, mais a quantificação plaquetária. Para realizar a avaliação da Imunoglobulina E total, utilizou-se 1 ml de soro sem anticoagulante, pelo método de quimioluminescência, através do aparelho INMULITE 2000 (Imunoassay System; Siemens, Califórnia, EUA). Para avaliar a quantidade de níquel circulante, foram utilizados 2 ml de soro, coletados em um tubo especial (tubo "Trace") por Espectrofotometria de absorção atômica (forno de grafite com corretor Zeeman). Exames de fezes de todos os indivíduos participantes foram realizados com o objetivo de verificar infestações parasitárias (ovos e larvas de helmintos e cistos protozoários) que pudessem alterar os valores do leucograma, em especial o número de eosinófilos. As fezes foram coletadas em recipiente estéril pelo método HPJ (Hoffman, Pons e Janer), com centrifugação e sedimentação espontânea, recentes e sem conservantes.

Além disso, os pacientes foram orientados para realizar a coleta sem contaminação do material. O material foi colocado na lâmina e avaliado em microscópio (Nikon, Alphaphot, 2Y52, New York, EUA). Todos os exames de laboratório foram realizados por um mesmo farmacêutico-bioquímico devidamente treinado, em um mesmo laboratório especializado.

*Avaliação gengival*

Os aspectos gengivais avaliados foram: características clínicas gengivais como a cor e volume e a presença ou não de sangramento gengival com o auxílio de uma sonda milimetrada IPC, preconizada pela Organização Mundial de Saúde. A sondagem foi realizada em três pontos distintos na face vestibular, lingual, mesial e distal dos elementos. Para tal avaliação utilizou-se o índice gengival de Löe (1967) que contempla os seguintes critérios:

0: Gengiva normal;

1: Inflamação leve: pequena modificação da cor; edema leve; sem sangramento à sondagem;

2: Inflamação moderada: aspecto avermelhado; edema leve; sangramento à sondagem;

3: Inflamação severa: aspecto avermelhado; edema bem evidenciado; ulceração; tendência ao sangramento espontâneo.

Avaliações da condição gengival dos participantes foram realizadas por um único examinador de forma cega e devidamente treinado e calibrado, antes do tratamento, durante o tratamento (com intervalo de 3 meses entre as avaliações) e 1 mês após a remoção dos aparelhos, totalizando 6 avaliações ($T_{0:}$ antes do início do

tratamento; $T_1$: 3 meses após o início do tratamento; $T_{2:}$ 6 meses após o início do tratamento; $T_3$: 9 meses após o início do tratamento; $T_{4:}$ 12 meses após o início do tratamento; $T_{5:}$ 1 mês após a remoção do aparelho). Em cada sessão era realizada adicionalmente (após a avaliação gengival) uma profilaxia com jato de bicarbonato objetivando controlar a variável "higiene bucal", de forma a não enviesar os resultados. Durante todo o tratamento ortodôntico, os indivíduos foram orientados para uma boa manutenção da saúde bucal.

As medidas para garantir o controle de infecção durante a execução dos exames clínicos, bem como as práticas de limpeza e esterilização de instrumental e eliminação de resíduos foram realizadas de acordo com o preconizado pelo Ministério da Saúde (Ministério da Saúde, 2000).

**Estudo piloto**

O estudo piloto teve como objetivo a avaliação do método e dos instrumentos escolhidos. Este estudo foi realizado para comparar parâmetros sanguíneos e características gengivais de pacientes ortodônticos alérgicos ao níquel com os de pacientes não alérgicos ao níquel. Os pacientes foram selecionados em uma amostra de conveniência de 96 pacientes. Depois de determinar a prevalência de alergia ao níquel, encontrada em 16 pacientes (17,2%), formaram-se dois grupos: 16 pacientes alérgicos e 16 pacientes não alérgicos. A aleatoriedade só foi utilizada para formar o grupo de pacientes não alérgicos, pois o total de pacientes alérgicos foram incluídos na amostra. Alergia ao níquel foi diagnosticada através do teste de contato, a condição gengival utilizando o índice gengival de Löe e as características sanguíneas através do

hemograma completo, incluindo a quantificação de níquel circulante e imunoglobulina E (IgE).

Após a avaliação dos resultados desta primeira etapa, foram feitas adaptações necessárias para o desenvolvimento do estudo principal, como por exemplo, a realização do teste de contato antes do início do tratamento ortodôntico, a utilização de braquetes com diferentes concentrações de níquel, nível de algumas citocinas inflamatórias, entre outras.

**Calibração do examinador para avaliação gengival**

A primeira fase consistiu em uma etapa teórica, quando foram apresentados os critérios dos índices, escores e a rotina a ser seguida durante o exame clínico da avaliação gengival. Esta etapa foi coordenada por um Periodontista, considerado padrão-ouro. Na segunda fase foram realizados exames clínicos, pelo examinador e pelo padrão-ouro, em 10 pacientes. Em caso de discordância na classificação do escore um novo exame era realizado. Os exames foram repetidos até que o padrão-ouro julgasse que os critérios já estavam fixados pelo examinador. Após um período 14 dias decorrido do primeiro exame foi realizado uma nova avaliação dos pacientes pelo examinador (Kappa intra-examinador: 0,80; Kappa inter-examinador: 0,88).

**Critérios de elegibilidade**

O critério de inclusão foi ter sensibilidade ao níquel.

Os critérios considerados para as perdas durante a pesquisa foram:

- Com o propósito de realizar avaliações gengivais com menor influência da má-higienização, seriam excluídos da amostra indivíduos que, por quaisquer motivos,

deixassem de comparecer aos controles periódicos de profilaxia executados pelo pesquisador;

- indivíduos que inadvertidamente removessem em prazo inferior a 48 horas, os testes de contato, uma vez que isso inviabilizaria a determinação da sensibilidade alérgica;

- indivíduos que não comparecessem ao laboratório para realização dos exames sanguíneos;

- indivíduos portadores de piercings intraorais e extraorais;

- pacientes do gênero feminino em período de gestação;

- pacientes não alérgicos ao níquel e pacientes que desistissem do tratamento ortodôntico qualquer que fosse a fase em que se encontrassem.

**Elenco de variáveis**

*Variável dependente*

A alergia ao níquel, detectada pelo teste de contato citado anteriormente, sobre as condições periodontais e sanguíneas dos pacientes submetidos à terapia ortodôntica foi avaliada através do Índice gengival de Löe (1967) e dos exames sanguíneos em todos os pacientes.

*Variáveis independentes*

As alterações gengivais e sanguíneas provenientes da alergia ao níquel nos pacientes da pesquisa serão associadas com as algumas variáveis independentes:

1. IgE
2. Níquel circulante
3. Leucócitos totais e diferenciais
4. Bastões
5. Índice gengival
6. Braquetes convencionais e níquel free

QUADRO 1 – Variáveis independentes: definição e categorização

| Variável independente | Definição da variável | Categorização |
|---|---|---|
| Índice gengival | Índice gengival de Löe (1967) | 0: Gengiva normal<br>1: Inflamação leve<br>2: Inflamação moderada<br>3: Inflamação severa |
| Hemograma | Determina a quantidade, qualidade e variedade de eritrócitos, leucócitos e plaquetas. | Será categorizado de acordo com a normalidade de cada componente |
| IgE | Anticorpo, Imunoglobulina E. Pacientes com doenças alérgicas tem altos níveis de IgE | Valor de Referência: Limite 87 UI/ml |
| Níquel circulante | Avaliação do níquel circulante no organismo. | Inferior a 4mcg/L |
| Braquetes | Convencionais e níquel free | Com níquel/baixa concentração de níquel |

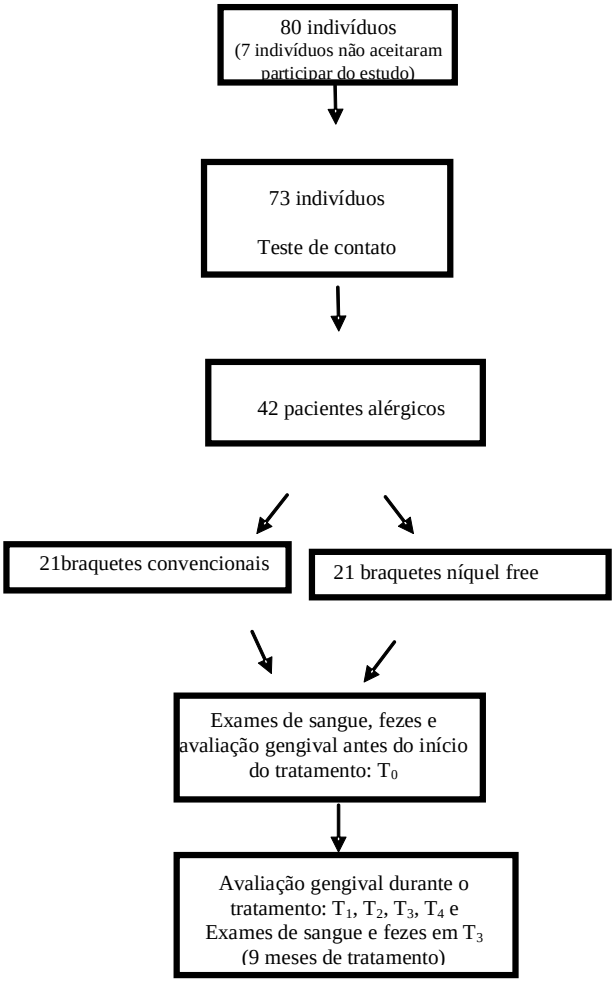
**Análise estatística**

Para analisar os dados foram utilizados recursos de computação para criar um banco de dados com as respostas de todos os participantes selecionados. Os dados desta pesquisa foram analisados de forma descritiva e analítica nos programas Microsoft Excel e o *Statistical Package for the Social Sciences* (SPSS for Windows, version 19.0, SPSS Inc., Chicago, EUA).

Os testes estatísticos Mann Whitney, Anova de Friedman, T pareado e não-pareado, Wilcoxon e Correlação de Pearson e Spearman foram utilizados.

Análises adicionais foram incluídas como a correlação entre o Índice gengival e o Leucograma (número de leucócitos totais e diferenciais), bem como entre o Índice gengival e IgE. Para o nível de significância foi considerado 5% ($p<0,05$) (Pereira, 2000).

## Fluxograma do estudo



UNIVERSIDADE FEDERAL DE MINAS GERAIS
Faculdade de Odontologia
Programa de Pós-Graduação em Odontologia
Av Pres.Antônio Carlos, 6627 - Pampulha
Belo Horizonte – MG 31.270-901
Tel: (31) 3409 2470 Fax: (31) 3409 24[illegible]
www.odonto.ufmg.br – odonto-posgrad@ufmg.br

Ata da Comissão Examinadora para julgamento da Tese de Doutorado em Odontologia, área de concentração em **Odontopediatria**, da candidata **Camila Alessandra Pazzini**.

Aos 09 de novembro de 2012, às 14:00 h, na sala de Pós-Graduação (3403) da Faculdade de Odontologia, reuniu-se a Comissão Examinadora, composta pelos professores Dr. Saul Martins de Paiva, Dr. Leandro Silva Marques, Dr. Camilo Aquino Melgaço, Dr. Flávio Ricardo Manzi, Dra. Tarcília Aparecida Silva e Dra. Elizabeth Maria Bastos Lages. O Professor Dr. Saul Martins de Paiva, Orientador da Tese, na qualidade de Presidente da sessão, apresentou a Comissão Examinadora e declarou abertos os trabalhos. À candidata foi dado o tempo de até 50 (cinquenta) minutos para fazer a exposição oral sobre o seu trabalho "**Alterações sanguíneas e gengivais em pacientes alérgicos ao níquel submetidos a tratamento ortodôntico com braquetes convencionais e níquel free**". Encerrada a exposição, foi iniciada a arguição, dentro do limite de tempo de 30 (trinta) minutos, pelos Professores Dr. Camilo Aquino Melgaço, Dr. Flávio Ricardo Manzi, Dra. Tarcília Aparecida Silva e Dra. Elizabeth Maria Bastos Lages, com limite de 30 (trinta) minutos para a resposta. Terminadas as arguições, o Presidente suspendeu os trabalhos por 10 minutos para que os examinadores pudessem decidir pelo resultado a ser dado à candidata. A Comissão Examinadora opta pela ...aprovação..... da candidata. Para constar, lavrou-se a presente ata, que vai assinada por mim, Dr. Saul Martins de Paiva, Presidente e pelos demais membros desta comissão examinadora. Belo Horizonte, 09 de novembro de 2012.

Dr. Saul Martins de Paiva
FO-UFMG - Orientador

Dr. Leandro Silva Marques
UFVJM - Co-Orientador

Dr. Camilo Aquino Melgaço
UNINCOR

Dr. Flávio Ricardo Manzi
PUC-Minas

Dra. Tarcília Aparecida Silva
FO-UFMG

Dra. Elizabeth Maria Bastos Lages
FO-UFMG

P348c 2012 T

Pazzini, Camila Alessandra
Condições sanguíneas e gengivais em pacientes alérgicos ao níquel submetidos a tratamento ortodôntico com braquetes convencionais e níquel free / 2012.
119 f.: il.
Orientador: Saul Martins de Paiva
Co-orientador: Leandro Silva Marques
Tese ( Doutorado)- Universidade Federal de Minas Gerais, Faculdade de Odontologia.
1.Hipersensibilidade - Teses. 2.Braquetes ortodônticos – Efeitos adversos -Teses. 2.Níquel – Efeitos adversos - Teses. I. Paiva, Saul Martins de II. Marques, Leandro Silva. III. Universidade Federal de Minas Gerais. Faculdade de Odontologia. IV. Título.

BLACK D047

Elaborada pela biblioteca da Faculdade de Odontologia da UFMG