NICKEL ALLERGY: BLOOD AND PERIODONTAL EVALUATION AFTER ORTHODONTIC TREATMENT

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ABSTRACT

The aim of this study was to assess periodontal status and blood parameters in orthodontic patients with nickel allergy one month after removal of brackets. Ninety-six randomly selected patients were initially evaluated. Allergy to nickel was diagnosed using a patch test. After determining the prevalence of subjects allergic to nickel, two groups were formed: 16 allergic (experimental) and 16 non-allergic (control) patients. Their periodontal status was determined regularly by a single, blinded, duly calibrated examiner using the Löe Index (GI) and their blood was tested (complete blood test, including nickel and IgE levels) after nine months of orthodontic treatment and again one month after removing the orthodontic appliances. Statistical analyses included paired and non-paired t-tests, Mann-Whitney, Wilcoxon, McNemar and linear trend chi-square tests ($p \leq 0.05$). Comparison of the values recorded during orthodontic treatment and one month after removing the appliances showed that in the allergic group there was significant increase in eosinophils ($p=0.046$), basophils ($p=0.001$) and monocytes ($p=0.002$), and decrease in number of bands ($p=0.000$), while in the control group, there was increase in lymphocytes ($p=0.039$) and decrease in segmented neutrophils ($p=0.030$) and IgE levels ($p=0.001$). In both groups, plasma nickel levels increased ($p=0.010$; $p=0.039$) and GI scores decreased. One month after removing the brackets, blood and periodontal parameters from patients with and without nickel allergy were similar.

Key words: *Allergy and immunology, blood, hypersensitivity, nickel, orthodontics.*

ALERGIA AO NÍQUEL: AVALIAÇÃO PERIODONTAL E SANGUÍNEA APÓS O TRATAMENTO ORTODÔNTICO

RESUMO

O objetivo do presente estudo foi avaliar a condição periodontal e os parâmetros sanguíneos em pacientes alérgicos ao níquel, um mês após remoção dos aparelhos. Noventa e seis pacientes selecionados aleatoriamente foram inicialmente avaliadas. Alergia ao níquel foi diagnosticada usando um teste de contato. Após a determinação da prevalência de alergia ao níquel, formaram-se dois grupos: 16 pacientes alérgicos (experimental) e 16 não alérgicos (controle). Condição periodontal foi diagnosticada através do Índice de Löe (IG). Parâmetros sanguíneos foram determinados por meio de um exame de sangue completo, incluindo a quantificação de níquel e níveis de IgE. Avaliações do estado periodontal foram realizados por um único examinador de forma cega, devidamente calibrado e amostras de sangue foram tomadas depois de nove meses de tratamento e um mês após a remoção dos aparelhos ortodônticos. Análise estatística utilizada foi testes t pareado e não pareado, Mann-Whitney, Wilcoxon, McNemar e qui-quadrado de tendência linear ($p \leq 0,05$). Em comparação com os valores observados durante o tratamento, o número de eosinófilos ($p=0,046$), basófilos ($p=0,001$) e monócitos ($p=0,002$) aumentou significativamente depois da remoção dos aparelhos ortodônticos, ao passo que o número de bastões ($p=0,000$) diminuiu entre os períodos no grupo alérgico. O número de linfócitos ($p=0,039$) aumentou no grupo controle e o número de segmentados ($p=0,030$) diminuiu. A diminuição dos níveis de IgE ($p=0,001$) entre os períodos ocorreu no grupo de controle. Níveis de níquel no plasma aumentou após a remoção de aparelhos ortodônticos em ambos os grupos ($p=0,010$; $p=0,039$). O IG diminuiu em ambos os grupos. Parâmetros periodontais e sanguíneos de pacientes com alergia ao níquel foram semelhantes aos não alérgicos um mês após a remoção dos aparelhos.

Palavras chave: *Alergia e Imunologia, Sangue, Hipersensibilidade, Níquel, Ortodontia.*

INTRODUCTION

Industrialization and modern life have contributed to an increase in dermal exposure to metals, increasing the incidence of allergies, especially to nickel[1], the so-called allergic contact dermatitis[2-4]. The prevalence of nickel allergy in the general population ranges from 8% to 17% in females and 1% to 5% in males[5]. Nickel is potentially allergenic

and capable of causing a late-phase, type IV hypersensitivity reaction characterized by signs such as gingival overgrowth, burning sensation in the mouth, metallic taste, angular cheilitis and labial desquamation in the oral cavity.[6]

Chemicals of small molecular weight (i.e., haptens) can irritate tissues by inducing the production of various pro-inflammatory and chemotactic molecules which are potentially allergenic when able to bind to proteins, such as immune response molecules.[7,8] 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 is assumed that the continuous release of small amounts of nickel into the epithelium could constitute an initiating factor of gingival overgrowth induced by orthodontic brackets[9]. The increasing incidence of periodontal diseases and Ni allergy on the one hand and the high need for orthodontic treatment on the other highlight the importance of improving the knowledge of Ni(II)-induced mechanisms[10] while maintaining strict control of hygiene during orthodontic treatment[11].

A recent systematic review[10] on hypersensitivity to nickel and orthodontic treatment pointed to serious methodological limitations such as inadequate description of the use and composition of braces, contact test standardization, lack of control groups and cross-sectional studies. An *in vitro* study suggested that nickel has various modifying effects on IL-1β-induced inflammatory processes, depending on the concentration, although the authors acknowledge that there are limitations in transferring their findings to an *in vivo* situation of the oral cavity [11].

A previous study by our group suggested that nickel is potentially capable of affecting periodontal status and blood cells in allergic patients during orthodontic treatment [12,13]. However, those results seemed to be more related to a local inflammatory response than to a systemic allergic reaction. Moreover, after conducting those studies, another question arose of whether the cumulative effect of nickel during orthodontic treatment is reversed after brackets are removed or whether there are significant lasting effects on periodontal status at the end of orthodontic treatment.

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

MATERIAL AND METHODS

Sample characteristics and study design

Procedures involving evaluation of this sample have been published elsewhere[12]. Briefly, ninety-six randomly selected orthodontic patients were initially evaluated and a case-control study was conducted. All subjects were white; 58 (60%) were female and 32 (40%) male; their ages ranged from 10 to 43 years. Allergy to nickel was diagnosed using a patch test. After determining the prevalence of patients allergic to nickel, two groups were formed: 16 allergic and 16 non-allergic patients.

Data collection

All 32 participants underwent full blood tests nine months after beginning orthodontic treatment [12] and again one month after the removal of the brackets, to determine leukogram, total immunoglobulin E (IgE) and circulating blood levels of nickel. Six mL of blood were vacuum collected (vacuo-time system) from each patient after fasting for 8 hours. For the blood count, 3mL of blood in a vacuum tube with the EDTA anticoagulant were analyzed on an automated hematology analyzer, using the ABX Micros CRP device (OT-CT-OS-CS, France). Blood cell differential count was performed using a blood smear without anticoagulant, which was stained with Single Prov stain (NewProv – 1% solution of eosin methylene blue in cyclohexadiene), viewed under a microscope immersion objective for platelet count. For the evaluation of total IgE, 1 mL of serum without anticoagulant was analyzed using the chemiluminescence method on Immulite equipment. Two mL of blood were used to assess the amount of circulating nickel, collected in a trace tube for atomic absorption spectrophotometry (graphite Kiln with Zeeman corrector). The blood count determined number of leukocytes, basophils, eosinophils, myelocytes, metamyelocytes, bands, neutrophils, lymphocytes and monocytes.

Feces were examined to determine parasitic infestations (helminth eggs and larvae, protazoon cysts) which might affect the white blood cell count, especially the number of eosinophils, in order to control for confounding variables. Any subject with this type of alteration would be excluded from the study to prevent any camouflaging of the results. None of the subjects needed to be excluded.

The skin patch test was used for diagnosing nickel allergy. It is the most efficient method for

confirming the etiologic diagnosis of allergic-contact eczema. It consists of a 2 X 2 cm patch (Finn Chambers) which is attached to the patient's back at 2 points 10 cm apart after cleansing of the skin with cotton soaked in alcohol. Because of the extensive area involved, a suitable amount of the gel (standardized by the manufacturer) containing a 5% nickel sulfate antigen (solid petroleum jelly) (Epitest Ltd Oy, Tuusula, Finland) is left in contact for 48 hours. Patients were instructed to remove the patches if they experienced any reaction beyond what was expected, and to call the researchers in charge and seek care at the municipal medical emergency room. After 48 hours, the patches were removed, and only 1 reading was made, following the standards of the International Contact Dermatitis Research Group[14], 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 (+++).

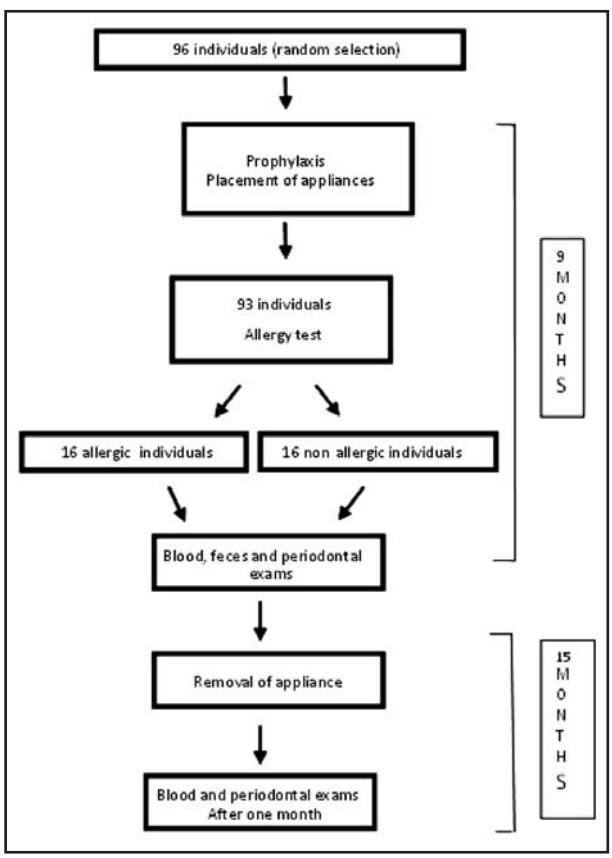

Fig. 1: Study Flowchart.

Periodontal status was assessed by a single, blinded, duly calibrated (Kappa > 0.90) examiner at regular three-month intervals over a period of 12 months (four evaluations altogether) during treatment, as described elsewhere[12]. Since each patient finished treatment at a different time, the final evaluation was standardized as one month after the treatment had been completed. Prophylaxis with bicarbonate spray was performed at each session (following periodontal 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[15] with a standardized millimeter probe, which takes into account qualitative changes in the gingival tissue. The Löe index used 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. This index was chosen because we have used it previously in this sample and thus maintain the same standard of evaluation, it is easy to perform, provides good reproducibility and its use is well established in the literature[16-18]. Morelli® brackets (Sorocaba, São Paulo, Brazil) were attached. Fig. 1 provides a flowchart illustrating the study design and sequence of procedures.

Statistical analysis

Statistical analysis involved t-tests, paired t-tests, Mann-Whitney and Wilcoxon tests for the intergroup/intragroup comparisons of blood components recorded after nine months of orthodontic treatment and again 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 to compare gingival index (dichotomized as absence/presence) within each group between the two evaluation times. Differences were considered significant when $p \leq 0.05$.

Ethical considerations

All aspects of this study, including methods for obtaining informed consent and agreement from participants (parents/caregivers and adolescents), were independently reviewed and approved by the Human Research Ethics Board of the *Centro*

Universitário de Lavras (Brazil) under process number 0001.0.380.000-07. This study was conducted in accordance with the principles for medical research involving human subjects set forth in the Helsinki Declaration. Collected data remained anonymous and confidential.

RESULTS

The following changes occurred between month nine of orthodontic treatment and one month after removal of the orthodontic appliances. In the allergic group, the number of eosinophils, basophils and monocytes increased significantly, whereas the number of bands decreased ($p<0.05$) (Table 1). In the control group, the number of lymphocytes increased, while IgE levels and number of segmented neutrophils and neutrophils decreased ($p<0.05$) (Table 1). In both groups, plasma nickel levels increased ($p<0.05$) (Table 1), while Gingival Index (GI) scores decreased ($p<0.05$) (Table 2).

Figs 2 and 3 show the periodontal condition of patients with and without nickel allergy, respectively.

DISCUSSION

After conducting the first experiment[12], one question remained: Would the periodontal and blood conditions be similar between allergic and non-allergic patients after removing the brackets despite

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±sd	After mean±sd	D-A p-value	During mean±sd	After mean±sd	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]
Segmented (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.
D-A: Comparison of mean values observed "during treatment" and "after brackets removal"

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

TESTS	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) withineach group comparing times: during and after orthodontic treatment. (Experimental p=**0.016***; Control p=0.070).
*($p<.05$); AL= allergic; NAL= Non allergic.

Fig. 2: Periodontal condition of allergic patient.

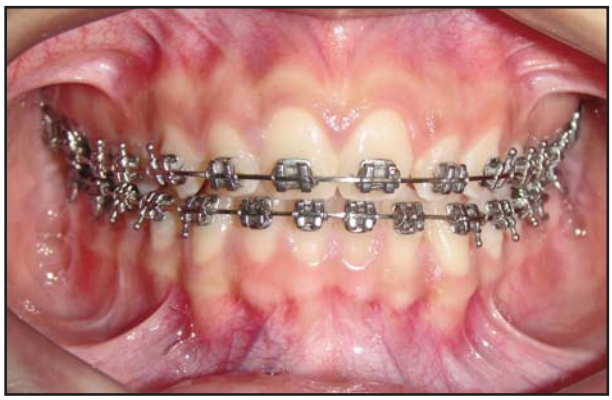

Fig. 3: Periodontal condition of non-allergic patient.

the possible cumulative effects of orthodontic treatment? We collected data again to answer this question.

Current data indicate a significant reduction in GI and band counts after the removal of orthodontic appliances, showing that periodontal and blood alterations tend to disappear. As orthodontic appliances hamper oral hygiene, dental biofilm accumulates more easily on tooth surfaces and appliances in most patients and the consequent periodontal disease leads to an increase in neutrophils[19]. Once the appliance is removed, the conditions favoring the formation of biofilm are no longer present and there is a consequent reduction in gingival index scores and amount of bands. Thus, the reduction in the number of bands may be explained by the decrease in bacterial accumulation and consequent decreased inflammatory reaction[20].

Gingival index scores were higher in the experimental group at both times. In addition to being 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[21]. The gingival epithelium is the first barrier which comes into contact with corrosive materials such as Ni (II) and bacteria[22]. This suggests a cumulative effect of nickel throughout orthodontic treatment, with nickel potentially influencing periodontal status of allergic orthodontic patients[23].

IgE levels decreased between the evaluations performed during treatment and after the removal of the orthodontic appliances in the control group. Circulating levels of nickel increased between evaluations in both groups, although within normal limits. Other studies have reported serum nickel levels to increase up to 5-fold during the 6-week post-closure period, and mean concentrations of nickel in serum to have returned to baseline levels within 4-6 months[24].

An analysis of white blood cells in allergic and non-allergic patients during and after treatment (Table 1) showed an increase in eosinophils, monocytes and basophils. We hypothesized that these results were not related to the removal of the appliances, but rather to the extensive 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, with a characteristic cell and molecule population through a pathway dependent on STAT-6 and GATA-3[25]. In our study, lymphocytes, eosinophils and IgE increased in both allergic and non-allergic patients, indicating the onset of a T_H2 immune response. These results corroborate findings in other studies, which observed the systemic response to nickel[26]. 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[27-29].

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[30]. However, in our study, allergic patients exhibited a greater response than non-allergic patients because they were more

sensitive to the allergen (nickel). Only the allergic group exhibited a significant increase in eosinophils, monocytes and basophils (Table 1). Similarly, the number of lymphocytes also increased after braces were removed. This may be explained by the fact that persons who are allergic to nickel have few or no specific suppressor T cells, which regulate the number of leukocyte populations[31].

Studies on hapten-induced contact hypersensitivity, which represents the classic model of a T cell-mediated hypersensitivity reaction, show that a strong inflammation response on the skin is elicited well before the activation of nickel-specific T cells[32]. A broad spectrum of chemokines is released, including Regulated on Activation, Normal T cell Expressed and Secreted (RANTES), which can play a fundamental role in histamine and serotonin generation and trigger human mast cell degranulation[33].

The results of our 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 to treat patients who exhibit compromised periodontal health. Moreover, the results of our study demonstrate that the allergic effect of conventional braces is reversed after the removal of the appliances, and metal ions released from appliances should not be cause for concern in utilizing the appliance[34].

In conclusion, no difference was found in blood or periodontal parameters between orthodontic patients with and without allergy to nickel one month after removing brackets.

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