



Oral nickel exposure may induce Type I hypersensitivity reaction in nickel-sensitized subjects ☆☆☆

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ABSTRACT

Background: Little is known about the clinical and immunological changes in the nickel allergic patients with systemic symptoms. We aimed to evaluate T helper cell responses of patients with different clinical presentations due to nickel.

Methods: Patients having various allergic symptoms and positive patch test results to nickel and 20 controls underwent skin prick tests with nickel. IL-10, IL-4, IL-5 and IFN-gamma were measured in the culture supernatants of PBMC stimulated by nickel during lymphocyte proliferation test (LTT).

Results: 69 patients (56 female, mean age: 49.2 ± 13.1), 97% having nickel containing dental devices and 20 controls (8 female, mean age 34.9 ± 12.06) were evaluated. Skin prick tests with nickel were positive in 70% of the patients ($p < 0.001$), being significantly higher in the patients with urticaria/angioedema ($p = 0.02$). The LTT stimulation index ($p < 0.0001$), IL-4 ($p = 0.002$), IFN-gamma ($p = 0.01$), IL-5 ($p = 0.04$) and IL-10 ($p = 0.003$) were higher in the patient group. LTT stimulation index, IL-4 and IL-10 were significantly elevated in patients having urticaria, angioedema and respiratory symptoms when compared to those who had only oral symptoms or systemic dermatitis ($p = 0.004$, $p = 0.002$ and $p = 0.01$, respectively).

Conclusion: This study suggests the presence of Type I hypersensitivity in addition to a Type IV immune reaction in patients with chronic systemic symptoms related to nickel. Nickel containing dental alloys and oral nickel intake seem to trigger systemic symptoms in previously nickel sensitized patients.

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1. Introduction

Since its discovery, nickel has been one of the most widely used metals and is among the most common contact allergens [1]. About 20% of the female and 6% of the male population are sensitized to nickel [2]. Epidemiological studies have revealed that sensitization to nickel is, in general, the result of a prolonged direct epidermal exposure, especially after skin piercing [3]. The clinical picture of nickel hypersensitivity is usually eczematous, but non-eczematous reactions can also be seen. The non-eczematous pattern of nickel hypersensitivity is broad and includes a wide number of skin reactions. Erythema multiform-like lesions, lichenoid, granuloma annulare and vitiligo-like reactions have been reported [1].

Contact dermatitis elicited by nickel is a Type IV delayed hypersensitivity immune response and is usually diagnosed by skin-patch tests [2]. Nickel induced Type I hypersensitivity eliciting an urticarial response has rarely been reported. Both immediate contact urticaria and allergic contact dermatitis were observed in one patient as a result of nickel-containing stainless steel devices [4]. We recently reported a patient who was experiencing throat swelling due to nickel containing dental alloys and recovered with the removal of the nickel alloy and a low-nickel diet [5].

When a nickel sensitized individual is exposed systemically to the metal (per os, respiratory, or parenteral), a flare-up of earlier eczema sites can occur and this phenomenon is generally called systemic contact dermatitis. Beyond an eczema flare, a generalized maculopapular rash and vasculitis-like skin lesions can be found, along with systemic symptoms such as headache, malaise, diarrhea, fever, and arthralgia [1]. Furthermore, allergic contact mucositis (ACM) has been recently described as a systemic nickel hypersensitivity and shown to be identifiable by the oral mucosa patch test (omPT) [6]. The exact mechanism of systemic contact dermatitis is not well understood. It may indicate T cells resting at the area of previous contact dermatitis or homing in

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on the site upon a systemic allergen exposure. Circulating immune complexes together with non-specific cytokine release by hapten stimulation are potentially responsible for the generalized reactions [7].

The scientific basis for lymphocyte transformation test (LTT) has been well-established in recent years, and its usefulness has been demonstrated in various diseases and with many different drugs. It has also long been used for the diagnosis of metal allergies. A particular advantage of this in vitro system measuring T-cell reactions to antigens is its potential to detect both the conductor as well as the key players in the immune system [8]. Assessing the metal-induced cytokine profile using the in vitro stimulation of peripheral lymphocytes with metal salts has improved the understanding of metal allergy mechanisms. Stimulation with nickel, cobalt, and chromium has been shown to induce specific patterns of cytokine secretion in lymphocyte cultures from metal-allergic patients, which involve both Th1- and Th2-type cytokines [9–11]. Although this cytokine pattern was attributed to only systemic contact dermatitis caused by nickel, there is no knowledge about the T cell properties in other types of manifestations due to nickel sensitization. Therefore, we aimed to evaluate T helper cell responses in lymphocyte cultures of patients with different clinical characteristics due to a nickel allergy suggesting a Type I hypersensitivity.

2. Materials and methods

2.1. Patient characteristics

The patient group consisted of 69 patients who were referred to our allergy department for various symptoms and determined to be sensitive to nickel sulphate according to their patch test results (Chemotechnique Diagnostics, Vellinge, Sweden). A group of 20 healthy subjects constituted the control group. Patient characteristics such as atopy, physical symptoms, history of ear piercing, history of dental cast alloys, and familial history of nickel allergy were determined through a detailed history.

2.2. Skin prick tests

Thirty-nine patients who accepted to be tested underwent skin prick tests with common aeroallergens (ALK Abell, Hørsholm, Denmark) and also with a nickel sulphate suspension (10 mg/ml, freshly prepared) to evaluate an immediate allergy to nickel. The aeroallergens and nickel sulphate suspension were dropped on the volar side of forearms of the patients and then a prick test lancet was used to penetrate the skin through the drops. A positive control test with histamine and a negative control test with saline were also applied simultaneously by the same method. The tests were interpreted after 15 min with test results considered positive if it raised a wheal and flare reaction 3 mm larger in diameter than that of the negative control or equal or more than half of the positive control. Prick tests with nickel sulphate suspension were also performed in 10 healthy controls to exclude an irritation reaction.

2.3. Lymphocyte transformation test (LTT)

LTT was performed in 44 patients and 8 healthy control subjects who accepted to have laboratory investigation. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood utilizing a Ficoll-Paque (Sigma Chemical, St Louis, MO) gradient. In vitro proliferation assays were performed as previously described by Pichler and Tilch [8], with some modifications.

PBMCs (1×10^6 /ml) were cultured in wells containing 100 μ l RPMI-1640 (Sigma Chemical) medium supplemented with 10% autologous plasma in a round-bottom microtiter plate. Cultures were performed in triplicate at 37 °C and 5% CO₂ for 5 days. Nickel sulphate (Merck, Frankfurt, Germany) concentrations were chosen as 10, 25, 50, 75, 100, 200 mM for lymphocyte stimulation as described by Lindemann [12]. As positive and negative controls, cells in triplicate were also incubated in the presence of 5 μ g/ml phytohemagglutinin (PHA), and in the

absence of these agents, respectively. Lymphocyte proliferation was measured using MTT assay (viable cells) as described by Mossman [13] and was corrected by counting dead cells with a cell counter (Countess-Invitrogen, Carlsbad, CA) after the exclusion of Trypan blue. The stimulation index (SI) was defined as the ratio of mean positive wells/mean negative wells. The mean LTT was considered as positive when the SI was equal to 2 or higher.

2.4. Cytokine determination

On the fifth day, supernatants were obtained from the wells with the highest lymphocyte proliferation in the nickel-stimulated PBMC microcultures of the patients and control subjects. The levels of IL-10 (Diacclone Gen-Probe, Besancon Cedex, France) IL-4 (Diacclone Gen-Probe), IL-5 (Diacclone Gen-Probe) and IFN- γ (Bender MedSystems, Vienna, Austria) were measured in the culture supernatants by ELISA according to the manufacturer's protocol.

2.5. Statistical analysis

The statistical differences in cytokine levels and SI between controls and patients were determined using unpaired t-test. The correlation between SI and cytokine levels was evaluated by the Pearson correlation test. Significance was evaluated as two-tailed. Nickel sulphate patch and skin prick test results of the two groups were compared using Chi-square test. The statistical differences in cytokine levels and SI among three different symptomatic patient groups (oral symptoms, systemic dermatitis and urticaria/angioedema) were calculated with one-way ANOVA test. Significance was evaluated as one-tailed. The p values < 0.05 were considered significant. All statistics were performed using the GraphPad Prism package version 6.0 (GraphPad Software, San Diego, CA).

3. Results

The clinical data of 69 patients (56 female, mean age: 49.2 ± 13.1) and 20 healthy control subjects (8 female, mean age 34.9 ± 12.06) were evaluated. Demographic properties and the clinical characteristics of the study patients are shown in Table 1. Various oral symptoms including lichen, mucosal wounds and burning mouth were reported by 47 patients (68%) of whom 18 concomitantly complained of various skin symptoms. Skin symptoms without oral complaints and respiratory

Table 1
Characteristics of study patients.

Features	Patients N = 69
Gender	56 F
Age	49.2 ± 13.1
Symptoms	
Oral symptoms	29 (42%)
Oral + skin symptoms	18 (26%)
Only skin symptoms	15 (21%)
Types of skin symptoms (all)	
Pruritus	14 (20.3%)
Urticaria/angioedema	17 (24.6%)
Contact dermatitis	7 (10.1%)
Respiratory complaints (laryngeal swelling, hoarseness)	7 (10%)
Symptom increase with nickel rich food	27 (40%)
Symptom decrease with a low-nickel diet	25/27 (92.6%)
Atopy	20 (28%)
Asthma	5 (7.2%)
Complaints related to ear piercing	45 (65%)
History of dental devices	67 (97%)
Symptom duration	
One year	26%
Three years	26%
Five years	17%
>Five years	31%

symptoms were described by 15 (21%) and 7 (10%) patients, respectively. According to the results of patch tests with dental materials, the patients with skin and respiratory symptoms were advised to follow a low nickel diet. After following the diet for one month, a significant symptom relief was observed in the majority of the patients. Seven patients with recurrent respiratory symptoms due to laryngeal edema, which was confirmed by an ear, nose and throat (ENT) examination, improved after the removal of their nickel-containing dental casts.

Skin prick tests with nickel sulphate were positive in 27 out of 39 patients (70%), while they were positive in only three of the control cases ($p < 0.001$). Skin test positivity was higher in the patients with urticaria and angioedema when compared to those with oral symptoms or pruritus and systemic dermatitis ($p = 0.02$). Patch tests were positive for all of the patients and for two of the control subjects (Table 2).

The LTT stimulation index was significantly higher in the patient group than in the control group ($p = 0.001$). Also, cytokine levels as IL-10 ($p = 0.003$), IFN- γ ($p = 0.01$), IL-4 ($p = 0.002$), and IL-5 ($p = 0.04$) were higher in the patient group when compared to the control group (Fig. 1, Table 2).

LTT stimulation index of patients was well correlated with some of the cytokine levels ($p < 0.0001$ for IL-4, $p = 0.04$ for IFN- γ , $p = 0.0007$ for IL-10) (Table 3). There were also significant correlations between atopy and the LTT stimulation index ($p < 0.002$), IL-4 ($p = 0.01$) and IFN- γ levels ($p = 0.009$).

Considering all nickel patients divided into groups according to symptoms, LTT stimulation index, IL-4 and IL-10 were significantly elevated in the patients ($n = 10$) having urticaria, angioedema and respiratory symptoms when compared to those who had oral symptoms ($n = 15$) or systemic dermatitis ($n = 12$) ($p = 0.003$, $p = 0.01$ and $p = 0.01$ respectively) (Table 4).

4. Discussion

The results of the current study suggest that oral nickel exposure through dental alloys or oral intake may cause various and sometimes serious systemic hypersensitivity reactions. The nature of some symptoms of our patients including chronic urticaria, angioedema, respiratory complaints and even laryngeal edema, along with the characteristics of the cytokine profile and skin test results led us to consider that the underlying mechanism could be a Type I reaction in addition to Type IV immunologic mechanism. Clinically, the quick resolution of the symptoms upon removal of the nickel-containing dental devices and low nickel diet supports this conclusion.

It is well known that nickel hypersensitivity is an example of hapten-mediated allergic contact dermatitis [14]. This hypersensitivity is proposed to begin with the binding of nickel ions (Ni^{2+}) to larger proteins and converting self-antigens into highly immunogenic “foreign” peptides. Haptens penetrating the epidermis bind to the peptide major histocompatibility complex (MHC) molecules of cutaneous dendritic cells,

which migrate to draining lymph nodes and present the antigen to hapten-specific naive T cells. These T cells are then expanded and, on re-exposure, drive an inflammatory reaction within the skin. Both CD4⁺ and CD8⁺ Ni^{2+} -specific T cells can be detected in the blood of patients with a nickel allergy and these cells secrete high levels of interferon- γ on exposure to Ni^{2+} in vitro. Besides this mechanism, it was shown that Ni^{2+} directly binds to human Toll-like receptor 4 and that this is sufficient for nickel sensitization [14,15]. However, some reports demonstrate that nickel-sensitive patients may represent eczematous lesions at sites other than those that have been in direct contact with nickel-plated items [16]. Accordingly, systemic nickel sensitivity, possibly caused by ingested nickel, has been proposed [17]. The results of a very recent study are quite consistent with this concept. The researchers have shown a significant increase in the number of circulating lymphocytes in nickel sensitive patients after the oral mucosal patch test with nickel [18]. Apart from eczema or systemic contact dermatitis, very few reports show nickel-induced contact urticaria, suggesting a Type I reaction [4]. In our series, cutaneous symptoms consisting of urticaria/angioedema and pruritus were frequently resolved after a low nickel diet or removal of dental devices. These observations and the skin prick test positivity in 70% of sensitized patients clinically suggested that nickel may also cause a Type I sensitivity.

Some studies propose an immediate type reaction against nickel. Shirakawa et al. proposed that nickel-specific IgE antibodies may play a role in the etiology of occupational nickel related asthma caused by nickel sulphate inhalation [19]. Similarly, Spanish researchers investigated patients with work-related asthma symptoms and proposed that nickel salts may induce asthma symptoms by either non-immunologic or immunologic mechanisms including IgE mediated reactions [20]. In the abovementioned study, Di Tola et al. observed that, in spite of the moderately increased number of circulating B lymphocytes after the oral mucosal nickel patch test in nickel sensitive patients, total IgE serum concentrations were not increased [18]. They proposed that this finding can exclude the possibility of an IgE-dependent immediate hypersensitivity reaction and indicate that nickel may act a mast cell discharger. Although this hypothesis could be within the bounds of possibilities, the existence of an IgE-mediated immune mechanism cannot be ruled out unless nickel-specific IgE levels were measured.

Formerly, nickel was postulated to induce only a Th1 cell response and a related cytokine pattern. Later, researchers demonstrated that nickel was also able to stimulate Th2 cells [10,17,21]. Borg et al. demonstrated the increase of IL-4 and IL-5 but not IFN- γ in the PBMC culture supernatants of nickel-sensitized contact dermatitis patients after nickel stimulation [10]. Jensen et al. investigated the characterization of lymphocyte subpopulations and cytokine profiles in the peripheral blood of nickel-sensitive individuals with systemic contact dermatitis after oral nickel exposure [21]. They reported that nickel-sensitive individuals who clinically reacted to an oral challenge with nickel had elevated levels of IL-5 in the serum, indicating an activation of Type 2 T lymphocytes in the peripheral blood. In order to reveal the nature of T-lymphocytes in the peripheral blood of Ni-allergic individuals with local and systemic contact dermatitis, Ricciardi et al. evaluated IL-12 and IL-10 in the peripheral blood of the patients and found that the patients with nickel-induced local and systemic dermatitis had higher levels of IL-12 and IL-10 levels, the latter being non-significant. They proposed that nickel-specific T lymphocyte clones of nickel allergic patients produce both Th1 and Th2 type cytokines [17]. According to our results, Th1 cytokine IFN- γ , Th2 cytokines IL-4, IL-5 and an immunoregulatory cytokine IL-10 were significantly higher in the supernatants of nickel sulphate stimulated peripheral blood lymphocytes of the nickel-sensitive patients when compared to control subjects. These findings are concordant with the results of previous studies [10, 21] and in our opinion, may explain both urticarial and laryngeal symptoms of some patients. Although this cytokine profile can also reflect the underlying atopic status of patients, one limitation of our study is the lack of evaluation of cytokine release from unstimulated lymphocytes.

Table 2
Patch test, skin prick test, LTT and cytokine levels of patients and control subjects.

	Patients	Controls	p
Nickel sulphate patch test results	All positive n = 39	2 positive n = 20	<0.001
Nickel sulphate skin prick test results	27 positive n = 39	3 positive n = 10	<0.001
	Patients n = 44	Controls n = 8	p
LTT stimulation index ^a	2.63 ± 0.28	1.08 ± 0.08	0.001
IL-10	105.6 ± 13.24	39.0 ± 9.04	0.003
IFN- γ	0.99 ± 0.16	0.27 ± 0.13	0.01
IL-4	2.44 ± 0.15	1.09 ± 0.31	0.002
IL-5	0.11 ± 0.02	0.06 ± 0.00	0.04

^a The LTT was considered as positive when the stimulation index was 2 or higher.

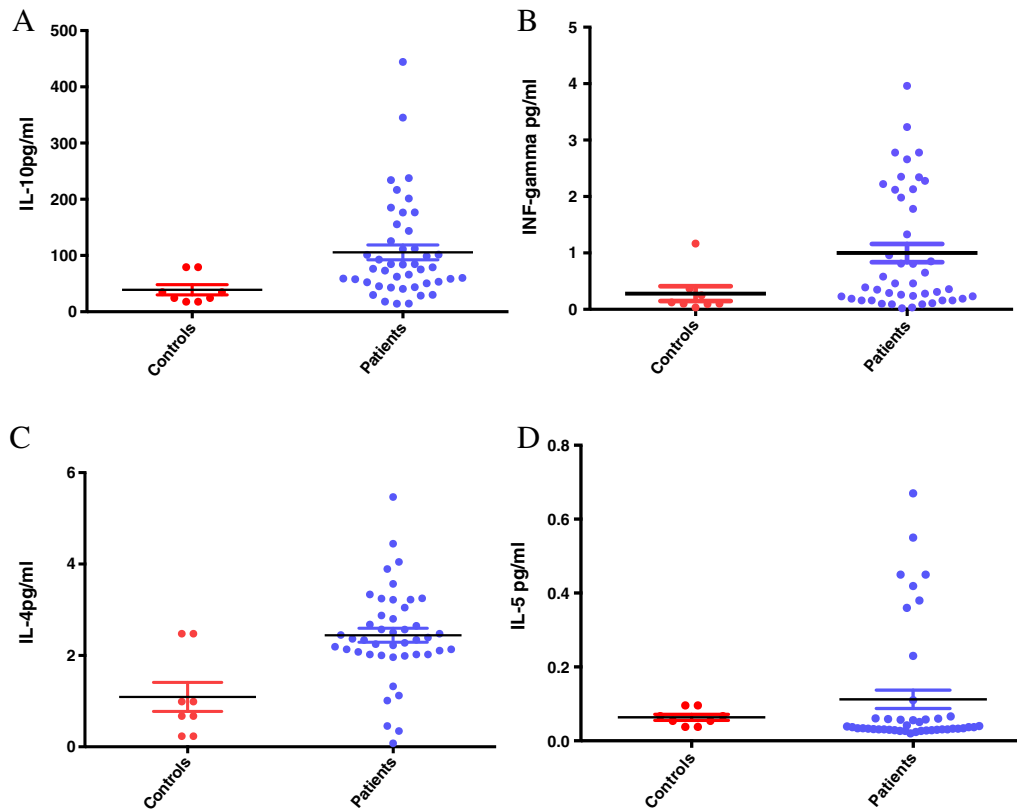


Fig. 1. Distribution of cytokine levels in nickel-allergic patients and control subjects. Horizontal bars represent medians. A) IL-10, ($p = 0.003$), B) INF-gamma, ($p = 0.01$), C) IL-4, ($p = 0.002$), D) IL-5, ($p = 0.04$) (For statistical analysis unpaired t-test was used).

Although initial nickel sensitization usually occurs with nickel earrings, the subsequent nickel exposure route is important in the development of systemic hypersensitivity. While most of the patients in our series had a local reaction history with nickel containing earrings, they did not experience systemic complaints until dental devices were placed in their mouths. It was claimed that the intraoral use of materials that contain components to which the individual is sensitized rarely cause allergic reactions [22]. In fact, some researchers showed that the use of intraoral appliances containing a known allergen may induce immunologic tolerance in non-nickel sensitized individuals [23,24]. But, if an early sensitization through a dermal contact, such as wearing an earring occurs first, such a tolerance cannot develop. On the other hand, the ability of a metal or alloy to induce and elicit allergic reactions is related to the pattern and mode of corrosion [22]. The fact that alloys of similar composition may have different corrosion behaviors can explain why some nickel sensitized patients develop systemic reactions after wearing dental alloys. Apart from dental devices, metallic orthopedic implants also have been considered to cause hypersensitivity reactions and consequently implant failure [25]. It was hypothesized that corrosion and wear of the alloys results in metal ion release, which may cause sensitization and even implant failure, owing to the acquired immune reactivity.

Table 3
Correlation of LTT index with cytokine levels of patients.

LTT stimulation index n = 44	p	r
IL-4	<0.0001	0.58
IL-10	0.0007	0.46
INF-gamma	0.04	0.26
IL-5	0.3	0.06

(Two-tailed Pearson correlation test was used for statistical analysis).

A significant proportion of our patients complained of systemic symptoms upon eating high nickel-containing food such as whole wheat, rye, cocoa, tea, and green salads. Particular attention has been paid to the influence of dietary nickel on the clinical course of nickel-induced dermatitis [26]. A group of researchers demonstrated that 1% of nickel sensitive individuals may react with systemic contact dermatitis to normal daily nickel exposure from drinking water and diet, i.e. 0.22–0.35 mg nickel [27]. Nickel oral hyposensitization was shown to be effective in patients with systemic nickel allergy syndrome by some open labeled studies [28–30]. Minelli et al. evaluated IL-13, IL-5 and INF-gamma release by peripheral blood mononuclear cells before and after the oral hyposensitization of 4 months with nickel and observed that all cytokines were significantly decreased after the treatment period [30]. Finally the first randomized double-blind, placebo-controlled trial demonstrated the efficacy of oral nickel hyposensitization in systemic nickel allergic patients, on especially gastrointestinal symptoms [31]. Although most of the publications related to systemic nickel allergy from eating nickel rich food mention systemic contact dermatitis flare ups, a significant number of our patients displayed urticaria and even laryngeal discomfort after such an exposure, suggesting a Type I

Table 4
Comparison of LTT index, cytokine levels among different symptom groups.

	OS n = 15	SD n = 12	U/A n = 10	p	F
LTT stimulation index	1.44 ± 0.27	2.21 ± 0.33	3.9 ± 0.84	0.003	6.61
IL-4	2.01 ± 0.23	2.29 ± 0.15	3.11 ± 0.34	0.01	4.93
IL-10	74.59 ± 10.59	89.20 ± 19	170.8 ± 37.37	0.01	5.19
INF-gamma	0.68 ± 0.20	1.13 ± 0.32	1.32 ± 0.41	0.3	1.20
IL-5	0.09 ± 0.03	0.11 ± 0.04	0.07 ± 0.03	0.2	0.75

OS; oral symptoms, SD; systemic dermatitis, U/A; urticaria/angioedema (for statistical analysis one-way ANOVA test was used).

allergy. As mentioned above, nearly all of those patients experienced relief after the cessation of high nickel-containing foods.

In conclusion, nickel induced hypersensitivity reactions can involve both Th1 and Th2 mediated mechanisms. Nickel sensitized individuals are at risk of systemic manifestations upon subsequent exposure through dental devices or high oral intake. In this regard, allergists should take into account a nickel allergy when faced with unresolved systemic allergies and collaborate with dentists for the management of nickel sensitized patients.

References

- [1] R. Darlenski, J. Kazanjiyeva, K. Pramatarov, The many faces of nickel allergy, *Int. J. Dermatol.* 51 (2012) 523–530.
- [2] T. Rustemeyer, B.M. von Blomberg, I.M. van Hoogstraten, D.P. Bruynzeel, R.J. Scheper, Analysis of effector and regulatory immune reactivity to nickel, *Clin. Exp. Allergy* 34 (9) (2004) 1458–1466.
- [3] T. Schafer, E. Bohler, S. Ruhdorfer, et al., Epidemiology of contact allergy in adults, *Allergy* 56 (2001) 1192–1196.
- [4] M.L. Walsh, V.H. Smith, C.M. King, Type 1 and type IV hypersensitivity to nickel, *Australas. J. Dermatol.* 51 (2010) 285–286.
- [5] S. Buyukozturk, A. Gelincik, M. Demirtürk, D. Erdoğan, L. Pur, B. Colakoglu, G. Deniz, Kuruca S. Erdem, Nickel dental alloys can induce laryngeal edema attacks: a case report, *J. Dermatol.* 40 (9) (2013) 740–742.
- [6] A. Picarelli, M. Di Tola, A. Vallecocchia, V. Libanori, M. Magrelli, M. Carlesimo, A. Rossi, Oral mucosa patch test: a new tool to recognize and study the adverse effects of dietary nickel exposure, *Biol. Trace Elem. Res.* 139 (2011) 151–159.
- [7] R.I. Nijhawan, M. Molenda, M.J. Zirwas, S.E. Jacob, Systemic contact dermatitis, *Dermatol. Clin.* 27 (2009) 355–364.
- [8] W.J. Pichler, J. Tilch, The lymphocyte transformation test in the diagnosis of drug hypersensitivity, *Allergy* 59 (2004) 809–820.
- [9] J.T. Minang, I. Arestrom, M. Troye-Blomberg, L. Lundeborg, N. Ahlberg, 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.* 146 (2006) 417–426.
- [10] L. Borg, J.M. Christensen, J. Kristiansen, N.H. Nielsen, T. Menné, L.K. Poulsen, Nickel-induced cytokine production from mononuclear cells in nickel-sensitive individuals and controls. Cytokine profiles in nickel-sensitive individuals with nickel allergy-related hand eczema before and after nickel challenge, *Arch. Dermatol. Res.* 292 (2000) 285–291.
- [11] E. Jakobson, K. Masjedi, N. Ahlberg, L. Lundeborg, A.T. Karlberg, A. Scheynius, Cytokine production in nickel sensitized individuals analysed with enzyme-linked immunospot assay: possible implication for diagnosis, *Br. J. Dermatol.* 147 (2002) 442–449.
- [12] M. Lindemann, J. Böhmer, M. Zabel, H. Grosse-Wilde, ELISpot: a new tool for the detection of nickel sensitization, *Clin. Exp. Allergy* 33 (2003) 992–998.
- [13] N.T. Mossman, Rapid colorimetric assay for cellular growth and survivals: application to proliferation and cytotoxicity assays, *J. Immunol. Methods* 65 (1983) 55–63.
- [14] B. Roediger, W. Weninger, How nickel turns on innate immune cells, *Immunol. Cell Biol.* 89 (2011) 1–6.
- [15] M. Schmidt, M. Goebeler, Nickel allergies: paying the Toll for innate immunity, *J. Mol. Med.* 89 (2011) 961–970.
- [16] J.W. Hsu, C. Matiz, S.E. Jacob, Nickel allergy: localized, id, and systemic manifestations in children, *Pediatr. Dermatol.* 28 (2011) 276–280.
- [17] L. Ricciardi, S. Gangemi, S. Isola, O. Fogliani, S. Saitta, F. Purello-D'ambrosio, Nickel allergy, a model of food cellular hypersensitivity, *Allergy* 56 (2001) 109–111.
- [18] M. Di Tola, M. Marino, R. Amodeo, F. Tabacco, R. Casale, L. Portaro, R. Borghini, A. Cristaudo, F. Manna, A. Rossi, O. De Pità, P. Cardelli, A. Picarelli, Immunological characterization of the allergic contact mucositis related to the ingestion of nickel-rich foods, *Immunobiology* 219 (7) (2014) 522–530.
- [19] T. Shirakawa, Y. Kusaka, K. Morimoto, Specific IgE antibodies to nickel in workers with known reactivity to cobalt, *Clin. Exp. Allergy* 22 (2) (1992) 213–218.
- [20] M. Fernández-Nieto, S. Quirce, J. Carnés, J. Sastre, Occupational asthma due to chromium and nickel salts, *Int. Arch. Occup. Environ. Health* 79 (6) (2006) 483–486.
- [21] C.S. Jensen, S. Lisby, J.K. Larsen, V.K. Veien, T. Menné, Characterization of lymphocyte subpopulations and cytokine profiles in peripheral blood of nickel-sensitive individuals with systemic contact dermatitis after oral nickel exposure, *Contact Dermatitis* 50 (2004) 31–38.
- [22] J.C. Setcos, A. Babaei-Mahani, L. Di Silvio, et al., The safety of nickel containing dental alloys, *Dent. Mater.* 22 (2006) 1163–1168.
- [23] K.J. Vreeburg, K. deGroot, M. von Blomberg, R.J. Scheper, Induction of immunological tolerance by oral administration of nickel and chromium, *J. Dent. Res.* 63 (1984) 124–128.
- [24] I.M. Van Hogstraten, K.E. Andersen, B.M. Von Bloomberg, B. Boden, D.P. Bruynzeel, D. Burrows, et al., Reduced frequency of nickel allergy upon oral nickel contact at an early age, *Clin. Exp. Immunol.* 3 (1991) 441–445.
- [25] S.S. Jakobsen, C. Lidén, K. Søballe, J.D. Johansen, T. Menné, L. Lundgren, D. Bregnbak, P. Møller, M.S. Jellesen, J.P. Thyssen, Failure of total hip implants: metals and metal release in 52 cases, *Contact Dermatitis* 71 (6) (2014) 319–325.
- [26] F. Purello-D'Ambrassio, G.F. Bagnato, B. Guarneri, et al., The role of nickel in foods exacerbating nickel contact dermatitis, *Allergy* 53 (Suppl. 46) (1998) 143–145.
- [27] C.S. Jensen, T. Menné, J.D. Johansen, Systemic contact dermatitis after oral exposure to nickel: a review with a modified meta-analysis, *Contact Dermatitis* 54 (2006) 79–86.
- [28] R.C. Panzani, D. Schiavino, E. Nucera, S. Pellegrino, G. Fais, G. Schinco, G. Patriarca, Oral hyposensitization to nickel allergy: preliminary clinical results, *Int. Arch. Allergy Immunol.* 107 (1–3) (1995) 251–254.
- [29] D. Schiavino, E. Nucera, C. Alonzi, A. Buonomo, E. Pollastrini, C. Roncallo, T. De Pasquale, C. Lombardo, G. La Torre, V. Sabato, V. Pecora, G. Patriarca, A clinical trial of oral hyposensitization in systemic allergy to nickel, *Int. J. Immunopathol. Pharmacol.* 19 (3) (2006) 593–600.
- [30] M. Minelli, D. Schiavino, F. Musca, M.E. Bruno, P. Falagiani, G. Mistrello, et al., Oral hyposensitization to nickel induces clinical improvement and a decrease in TH1 and TH2 cytokines in patients with systemic nickel allergy syndrome, *Int. J. Immunopathol. Pharmacol.* 23 (2010) 193–201.
- [31] M. Di Gioacchino, L. Ricciardi, O. De Pità, M. Minelli, V. Patella, S. Voltolini, V. Di Rienzo, M. Braga, E. Ballone, R. Mangifesta, D. Schiavino, Nickel oral hyposensitization in patients with systemic nickel allergy syndrome, *Ann. Med.* 46 (1) (2014) 31–37.