

Oral Mucosa Patch Test: A New Tool to Recognize and Study the Adverse Effects of Dietary Nickel Exposure

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Abstract On contact with the skin, nickel may cause allergic contact dermatitis, which can be diagnosed by an epicutaneous patch test. Nickel exposure via the intestinal mucosa can induce diarrhea, abdominal pain, and swelling. The aim of the present study was to investigate the relationship between these symptoms and nickel intake by means of a novel oral mucosa patch test. Eighty-six patients with intestinal symptoms related to ingestion of nickel-containing foods were submitted to epicutaneous and oral mucosa patch tests for nickel. All patients with positive oral mucosa patch test results were subject to a low-nickel diet and monitored over time. Skin lesions were observed in 33 out of 86 (38.4%) patients evaluated by the epicutaneous patch test. Mucosal lesions were seen in 53 out of 86 (61.6%) patients given the oral mucosa patch test. After 2 months of a low-nickel diet, 52 out of 53 (98.1%) patients showed an improvement of their symptoms. There is a significant correlation between response time of the oral mucosa patch test and the latency of symptoms after ingestion of nickel-containing foods. Consequently, the oral mucosa patch test can be used to recognize and study the adverse effects of dietary nickel exposure that could be defined as allergic contact mucositis. A low-nickel diet is also shown to be an effective treatment for this condition.

Keywords Allergic contact dermatitis · Allergic contact mucositis · Celiac disease · Epicutaneous patch test · Intestinal symptoms · Nickel · Low-nickel diet · Oral mucosa patch test

Introduction

Nickel (Ni) is a transition metal found in the environment, some drugs, cosmetics, detergents, jewelry, watches, keys and other commonly used metallic utensils. Contact with

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this ubiquitous element may easily occur by inhalation, ingestion, or through the skin. Its absorption depends on the solubility of the chemical form of Ni. In the blood, Ni is mainly bound to albumin. Its circulating levels reflect the degree of exposure to soluble compounds rather than insoluble salts or unabsorbed deposits of Ni metal in the lungs. The distribution of Ni in tissues depends on the type and length of exposure, with the kidneys as the primary route of elimination [1].

The main clinical manifestation caused by skin contact with Ni is allergic contact dermatitis (ACD), for which the epicutaneous patch test (ePT) is considered the gold standard for its diagnosis [2]. Similarly, Ni exposure through the intestinal mucosa following the ingestion of Ni-containing foods can result in other clinical conditions, including contact stomatitis [2, 3]. Several studies have also reported that even small amounts of Ni ingested with a normal diet are enough to flare up a preexistent ACD [4–6]. However, these observations are occasional and fragmentary, so the sensitivity to dietary Ni is a condition still poorly understood.

In our own experience, several intestinal symptoms not attributable to any known pathological condition, such as diarrhea, abdominal pain, and swelling, are often reported by patients after the ingestion of Ni-containing foods. The aim of the present study was to investigate the relationship between intestinal symptoms and Ni intake by a novel oral mucosa patch test (omPT), based on recent studies that have shown a close association between oral and intestinal mucosal compartments in patients with celiac disease, a complex autoimmune enteropathy induced by dietary gluten [7–9]. The omPT is described for the first time in the present study.

Materials and Methods

Patients

Eighty-six subjects (six male and 80 female), with a mean age of 40.8 years (range 14–66 years), were chosen from outpatients from our gastrointestinal unit (GU) during the period from December 2006 to May 2008.

Patients who presented intestinal and extra-intestinal symptoms related to the ingestion of Ni-containing foods were included in the study. Informed consent was obtained from each patient and all procedures in this study were in accordance with the ethical standards of the institutional committee for human experimentation.

The patients carried a detailed food diary and were given a full medical evaluation to assess the adverse effects of dietary Ni exposure, as well as the presence of other clinical conditions (e.g., celiac disease and lactase deficiency). The main clinical data of participants are given in Table 1. Both ePT and omPT for Ni were given to each patient. Those who tested positive for omPT were put on a low-Ni diet and were monitored over time to follow the course of their symptoms.

Patch Tests for Nickel

Epicutaneous Patch Test

A patch containing 5% solution of Ni sulfate ($\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$) in Vaseline was applied on the upper back of patients. After 48 h the patch was removed to look for any lesion or reaction in the test site, repeating the inspection at 96 h. The presence of erythema, edema, and/or vesicles on the test site was considered a positive result.

Table 1 Clinical Data of the Participants

Intestinal symptoms	Pts/total (%)	Extra-intestinal symptoms	Pts/total (%)	Other diseases	Pts/total (%)
Abdominal swelling	56/86 (65.1%)	Itching	15/86 (17.4%)	CD	18/86 (20.9%)
Abdominal pain	45/86 (52.3%)	Hives	11/86 (12.8%)	LD	13/86 (15.1%)
Diarrhea	23/86 (26.7%)	Headache	6/86 (7.0%)	T	5/86 (5.8%)
Constipation	9/86 (10.5%)	Erythema	5/86 (5.8%)	IgAD	3/86 (3.5%)
Stomatitis	5/86 (5.8%)	Dizziness	4/86 (4.7%)	OAs	3/86 (3.5%)
Dyspepsia	4/86 (4.7%)	Asthenia	2/86 (2.3%)	ACD to X	2/86 (2.3%)
Nausea	4/86 (4.7%)	Conjunctivitis	2/86 (2.3%)	P	2/86 (2.3%)
Meteorism	3/86 (3.5%)	Dermographia	2/86 (2.3%)	V	2/86 (2.3%)
Vomiting	3/86 (3.5%)				
Belching	2/86 (2.3%)				

Each patient presented at least one intestinal symptom referable to the ingestion of Ni-containing foods, but not everyone had an extra-intestinal Ni-related symptom or was suffering from another disease. The symptoms as well as other diseases with a frequency less than 2% were not included in this table

CD celiac disease, *LD* lactase deficiency, *T* thyreopathy, *IgAD* IgA deficiency, *OAs* other allergies (to eggs, wheat, and atopic dermatitis), *ACD to X* allergic contact dermatitis to metals other than Ni (cobalt and palladium), *P* psoriasis, *V* vitiligo

Oral Mucosa Patch Test

The omPT was performed inside the upper lip after removal of excess saliva with sterile gauze. Briefly, a 5-mm filter paper disk saturated with a 5% solution of Ni sulfate in Vaseline was applied on the test site and held in place by an adhesive transparent film (Tegaderm, 3M) that in turn, was covered with a small absorbent pad. After 2 h, the patch was removed and the site of application was closely observed to determine the presence of any lesion or reaction, repeating the inspection at 24 and 48 h. Even the occurrence of any general reaction was carefully evaluated. If the patients showed adverse effects before 2 h, the patch was immediately removed, the reaction time was annotated, and the presence of any local and/or general reaction was assessed. Specifically, the appearance of erythema, edema, and/or vesicles on the test site, as well as itching and dermographia, were considered as positive result.

Low-Nickel Diet and Follow-Up

The ubiquity of Ni makes it virtually impossible to eliminate it from the diet. To prevent the adverse effects caused by the ingestion of this element, a low-Ni diet is the only resource at hand. For this reason, patients showing positive omPT results received a list of foods containing high amounts of Ni (Table 2), with the recommendation to introduce these in very limited quantities and to not consume more than one Ni-containing food in the same meal [10]. All patients assessed the adherence to the low-Ni diet by means of a food diary compiled daily, while the clinical effects of the dietary Ni restriction were carefully evaluated after 2 months of treatment.

Statistical Analysis

The differences between qualitative data were assessed by means of the chi-square test, while differences between quantitative data were evaluated by the Student two-tailed *t* test

Table 2 Foods That Contain a High Amount of Nickel

Foodstuffs	Ni-containing foods
Fishes	Herring, mackerel, salmon, shellfish, tuna
Vegetables	Fresh and dried legumes (chickpeas, lentils, peanuts, peas, red kidney beans, soya beans and soy products), garlic, green leafy vegetables (spinach), onion, raw carrots, tomatoes
Fruits	Fresh and dried fruits (almonds, hazelnuts, walnuts)
Cereals	Buckwheat, maize, millet, oat, rye, whole grain, whole wheat
Beverages	Beer, coffee, initial water flow from the tap (especially in the morning), red wine, tea
Others	Baking powder, canned foods, cocoa and chocolate, foods cooked in stainless steel utensils (especially if acidic foods as tomatoes), gelatin, linseeds, marzipan, Ni-containing vitamin supplements, strong licorice, sunflower seeds

for independent data. Linear regression was used to evaluate the correlation index between quantitative data. In all statistical tests, the significance level was set at $p \leq 0.05$.

Results

Appearance of erythema, edema, and/or vesicles on the epicutaneous test site was observed in 33 out of 86 (38.4%) patients. The omPT resulted in erythema, edema, and/or vesicles on the test site (Fig. 1a–c), as well as itching and dermatographia (Fig. 1d) in 53 out of 86 (61.6%) patients. The percentage of patients presenting positive results was significantly higher ($p=0.004$) for the omPT than for those undergoing the ePT (Fig. 2).

The number of Ni-related symptoms was significantly higher in patients who gave positive results in either of the patch tests than that of patients with negative results ($p=0.010$ for the ePT and $p<0.001$ for the omPT). The number of Ni-related symptoms reported by patients with negative ePT was significantly higher ($p=0.002$) than for patients with negative omPT (Fig. 3).

Of the 86 patients included in the study, 18 (20.9%) and 13 (15.1%) had a previous diagnosis of celiac disease and lactase deficiency, respectively (Table 1). The percentage of patients with celiac disease or lactase deficiency giving positive results for both patch tests is not significantly different from that of patients with the same illnesses and with negative results (Table 3).

Among the 53 patients with positive omPT results, 39 (73.6%) showed local lesions and/or general reactions after 2 h. In eight cases (15.1%), the response to Ni was evident after more than 2 h and in six (11.3%) within 30 min from the test start (Fig. 4). A significant correlation ($p<0.0001$) was found between the omPT response time and the latency of symptoms described by the patients after ingesting Ni-containing foods (Fig. 5). Furthermore, after the omPT, all patients showing positive results reported a relapse of the intestinal and extra-intestinal symptoms previously registered in their clinical history.

All participants completed the study with excellent compliance of the low-Ni diet. After 2 months of dietary Ni exposure reduction, 52 out of 53 (98.1%) patients who had positive omPT results showed an improvement of their intestinal and extra-intestinal symptoms.

Discussion

In the present study, the omPT is introduced as a new tool to recognize and study the adverse effects of dietary Ni exposure.

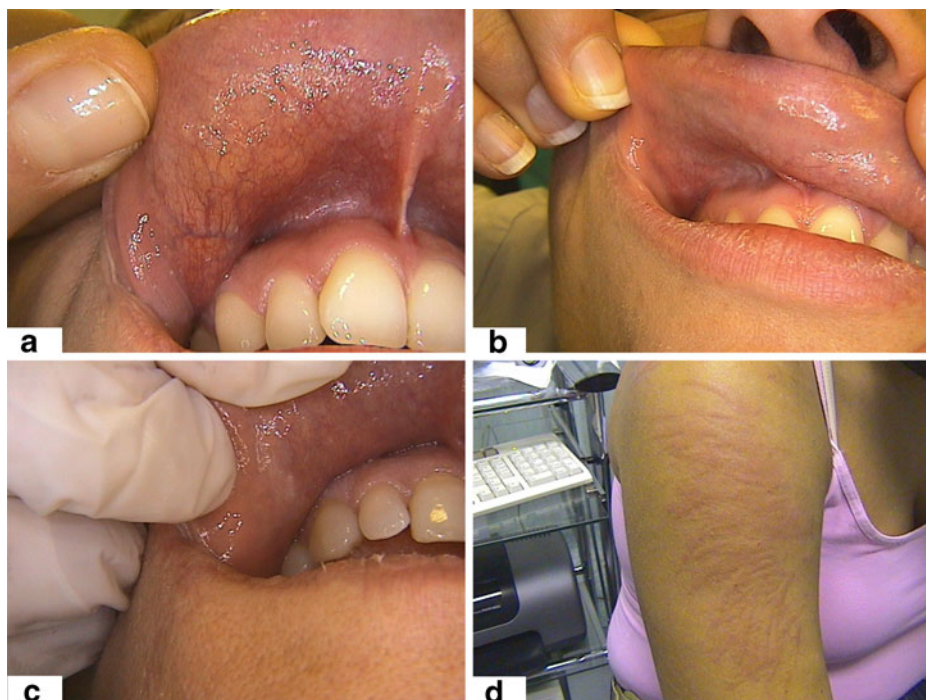
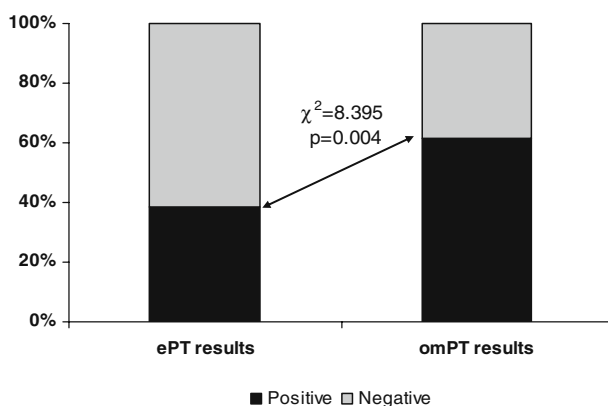


Fig. 1 Local and general reactions observable after the oral mucosa patch test for nickel. Appearance of erythema (a), edema (b), and vesicles (c) on the test site, as well as development of itching and dermographia (d) after performing the oral mucosa patch test in Ni-sensitive patients

A Ni patch is able to determine lesions of the oral mucosa including erythema, edema, and vesicle formation. This is consistent with reports of gingival abnormalities caused by Ni-based alloy prosthesis in orthodontic patients sensitive to Ni [11].

Recent studies have also shown a close association between oral and intestinal mucosa in patients with celiac disease, a complex autoimmune enteropathy induced by dietary gluten [7–9]. Consequently, the Ni-patch-induced oral mucosa lesions are probably related to bowel damage that occurs after ingestion of Ni-containing foods that in turn, causes the

Fig. 2 Percentage of patients showing positive patch tests for nickel. The percentage of patients presenting positive results for the epicutaneous and oral mucosa patch tests was plotted in this graph. The χ^2 and p values refer to chi-square test applied between these parameters. *ePT* epicutaneous patch test, *omPT* oral mucosa patch test



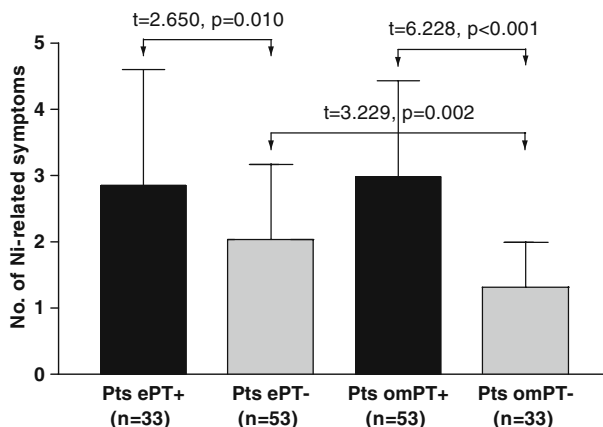


Fig. 3 Number of nickel-related symptoms in relation to the results of patch tests. The number of Ni-related symptoms reported by participants was plotted in this graph (mean $\pm$ SD) in relation to the results of epicutaneous and oral mucosa patch tests. The t and p values refer to the Student two-tailed t test for independent data applied between these parameters. *Pts ePT+* patients presenting positive epicutaneous patch test results, *Pts ePT-* patients with negative epicutaneous patch test results, *Pts omPT+* patients showing positive oral mucosa patch test results, *Pts omPT-* patients with negative oral mucosa patch test results

intestinal symptoms frequently reported by Ni-sensitive patients such as diarrhea, abdominal pain, and swelling.

A Ni patch may also cause development of general reactions such as itching and dermatographia, suggesting that the omPT can effectively identify Ni-sensitive patients. Furthermore, besides reinforcing the observations that Ni intake causes contact stomatitis [2, 3] and may trigger a preexistent ACD [4–6], our results show that the omPT is more sensitive than the epicutaneous version in recognizing the adverse effects of ingesting Ni-

Table 3 Percentage of Patients with Celiac Disease or Lactase Deficiency in Relation to the Results of Patch Tests for Nickel

	Pts	No pts	χ^2	p
CD				
Pts ePT + ($n = 33$)	8 (24.2%)	25 (75.8%)	0.104	0.747 (ns)
Pts ePT − ($n = 53$)	10 (18.9%)	43 (81.1%)		
Pts omPT + ($n = 53$)	9 (17.0%)	44 (83.0%)	0.754	0.385 (ns)
Pts omPT − ($n = 33$)	9 (27.3%)	24 (72.7%)		
LD				
Pts ePT + ($n = 33$)	4 (12.1%)	29 (87.9%)	0.091	0.762 (ns)
Pts ePT − ($n = 53$)	9 (17.0%)	44 (83.0%)		
Pts omPT + ($n = 53$)	10 (18.9%)	43 (81.1%)	0.849	0.357 (ns)
Pts omPT − ($n = 33$)	3 (9.1%)	30 (90.9%)		

The χ^2 and p values refer to chi-square test applied between these parameters

CD celiac disease, LD lactase deficiency, *Pts ePT+* patients presenting positive epicutaneous patch test results, *Pts ePT-* patients with negative epicutaneous patch test results, *Pts omPT+* patients showing positive oral mucosa patch test results, *Pts omPT-* patients with negative oral mucosa patch test results, *ns* not significant

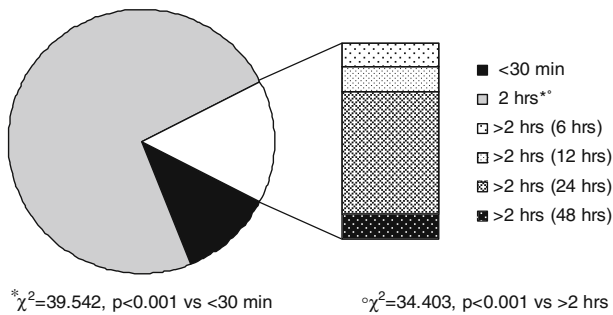


Fig. 4 Percentage of patients with different response times to nickel. The percentage of patients showing different response times to Ni during oral mucosa patch test (<30 min, 2 h, and >2 h) was plotted in this graph. The response times >2 h were split in other four time points (6, 12, 24, and 48 h). The χ^2 and p values refer to chi-square test applied between these parameters

containing foods in a population of symptomatic patients. In this regard, the significant portion of Ni-sensitive patients identified by the omPT, as well as the substantial clinical differences between positive and negative omPT patients further support this simple test as an effective diagnostic tool.

It is interesting to note that several individuals with positive omPT results are celiac disease or lactase deficiency patients, even if there is no evidence of a causal association. The high prevalence of celiac disease and lactase deficiency among the participants in this study could be simply due to a clinical bias because most of the patients that refer to our GU are suffering from these conditions. However, given that gluten-free diet usually include foods rich in Ni, the elective treatment for celiac disease could unmask a sub-clinical form of Ni-sensitivity. The latter hypothesis should be carefully considered in the monitoring of patients suffering from celiac disease.

The different response time to Ni among patients presenting positive omPT results is also of interest. Most patients react to Ni 2–48 h from the test start and are classified as late responders. There are also early responders, who have a response time of 30 min or less. It

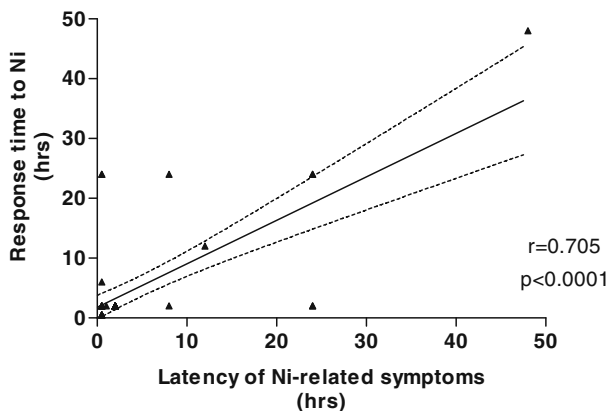


Fig. 5 Response time to nickel in relation to the latency of nickel-related symptoms. The response time to Ni observed during oral mucosa patch test and the latency of symptoms described by the patients after ingestion of Ni-containing foods were plotted in this graph. The r and p values refer to linear regression used to evaluate the correlation index between these parameters

is possible that these results are due to differences in the response mechanism. Late responders could react to Ni mainly by a delayed-type hypersensitivity or Th1 response, while early responders could do so predominantly by an immediate-type hypersensitivity or Th2 response.

For a long time, the scientific community has considered that the Ni-induced ACD response is of the Th1-type. By analyzing the cytokine secretion profile in peripheral blood of ACD patients, it has been shown that the majority of Ni-specific CD4⁺ T lymphocytes produce Th1 cytokines such as interleukin (IL)-2 and interferon (IFN)- γ [12]. Other authors have stressed the crucial role of Ni-specific CD8⁺ T cells in the pathogenesis of ACD [13–15]. Beyond the hypothesis based on the Th1-type mechanism, it has also been shown that Ni-specific CD4⁺ T lymphocytes from both peripheral blood and skin lesions of ACD patients are able to produce Th2 cytokines such as IL-4 and IL-5 [16–18]. Concurrently, other authors have shown that serological and urinary Ni levels correlate with the amounts of CD3⁺CD25⁺ and CD4⁺CD45RO⁺ T cells, CD5⁺CD19⁺ B cells, and IL-13 that may be found in peripheral blood of ACD patients in a pattern that reminds a Th2 response [19]. A more recent study has finally shown that Ni exposure leads to a concomitant production of Th1 (IFN- γ), Th2 (IL-4, IL-5, IL-13), and regulatory (IL-10) cytokines by peripheral blood mononuclear cells isolated from ACD patients [20].

Although the aforementioned studies are somewhat contradictory, they suggest that an imbalance among Th1, Th2, and regulatory pathways could be responsible for the immunological response that takes place in ACD patients during Ni exposure. Likewise, this mechanism could be taken as a model to explain the different response times to Ni observed in our investigation. Other studies will follow to fully elucidate the molecular bases of mucosal response to Ni.

Our data also highlighted that, in the course of omPT, patients showing positive results respond to Ni with the same latency than that described following ingestion of Ni-containing foods. Inversely, after a low-Ni diet, patients presenting positive omPT results show symptom relief. In this manner, we show for the first time a close relationship between Ni intake and intestinal symptoms commonly reported by Ni-sensitive patients. Finally, although previous data on the usefulness of dietary Ni restriction in ACD patients are slightly contradictory [21–23], we have shown that a low-Ni diet is effective in restoring the patient's general state of health.

In conclusion, the omPT can be used to recognize and study the adverse effects of dietary Ni exposure that could be defined as allergic contact mucositis (ACM). We suggest that patients with this condition be placed in a diet consisting of foods with the lowest possible Ni content over a reasonable period of time, during which the patient's response should be periodically evaluated. The high prevalence of Ni-sensitivity in North America and Western Europe, recently estimated to be 8.6% [24], stresses the relevance of the new acquisitions. However, the molecular role of Ni in ACM as well as in other intestinal disorders is still to be elucidated.

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