Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

Mercury and nickel allergy: risk factors in fatigue and autoimmunity

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Submitted: *July 15, 1999*
Accepted: *August 11, 1999*

Key words: **hypersensitivity; mercury; nickel; autoimmune thyroiditis; autoimmune polyglandular syndrome; chronic fatigue; MELISA®; lymphocyte stimulation; allergy**

*Neuroendocrinology Letters 1999; **20**:221–228 pii: NEL203499A07 Copyright © Neuroendocrinology Letters 1999*

Abstract

This study examined the presence of hypersensitivity to dental and environmental metals in patients with clinical disorders complicated with chronic fatigue syndrome. Three groups of patients were examined through medical history, dental examination, and by using a modified test of blast transformation for metals—MELISA®. The three groups consisted of the following: 22 patients with autoimmune thyroiditis with or without polyglandular autoimmune activation; 28 fatigued patients free from endocrinopathy; and 22 fatigued professionals without evidence of autoimmunity. As controls, a population sample or 13 healthy subjects without any evidence of metal sensitivity was included. Healthy controls did not complain of marked fatigue and their laboratory tests did not show signs of autoimmunity and endocrinopathy. We have found that fatigue, regardless of the underlying disease, is primarily associated with hypersensitivity to inorganic mercury and nickel. The lymphocyte stimulation by other metals was similar in fatigued and control groups. To evaluate clinical relevance of positive *in vitro* findings, the replacement of amalgam with metal-free restorations was performed in some of the patients. At a six-month follow-up, patients reported considerably alleviated fatigue and disappearance of many symptoms previously encountered; in parallel, lymphocyte responses to metals decreased as well. We suggest that metal-driven inflammation may affect the hypothalamic-pituitary-adrenal axis (HPA axis) and indirectly trigger psychosomatic multisymptoms characterizing chronic fatigue syndrome, fibromyalgia, and other diseases of unknown etiology.

Abbreviations

APS	Autoimmune Polyglandular Syndrome
CFS	Chronic Fatigue Syndrome
cpm	counts per minute
EBV	Epstein Barr Virus
HPA axis	Hypothalamic-Pituitary-Adrenal axis
LST	Lymphocyte Stimulation Test
MELISA	MEmory Lymphocyte ImmunoStimulation Assay
MHC	Major Histocompatibility Complex
MRI	Magnetic Resonance Imaging
PHA	Phytohemagglutinine
PWM	Pokeweed Mitogen
RPMI	Rosewell Park Memorial Institute (culture medium)
SI	Stimulation Index
TW	Tissue water

Introduction

Many patients with autoimmune polyglandular syndrome (APS) with manifestations of chronic fatigue syndrome (CFS) have hypersensitivity to heavy metals in the anamnesis.

In addition to clinical hypersensitivity, laboratory findings indicate a marked increase in the activation of the TH1 and TH2 subpopulations of lymphocytes [1, 2]. A variety of health disorders such as contact dermatitis, stomatitis and parodontitis, oral lichen planus, mild renal dysfunction, pneumoconiosis, resistance to antibiotics, and male infertility have been reported following exposure to heavy metals [3–9].

The human body is constantly exposed to metals and metal-containing compounds. Some metals are in trace amounts crucial for normal functioning while others exert toxic effects. The key factors governing the harmfulness of metals are the cumulative concentration, duration of exposure, and genetic susceptibility. Many harmless metals may become allergens or exert toxicity if administered on a chronic basis [10].

Metal-induced hypersensitivity in man is based upon the reaction of the allergen with the surface of memory T-lymphocytes sensitized to a specific allergen previously. Upon contact with the allergen, memory cells become activated and begin to produce lymphokines. The resulting inflammation can occur in the skin or elsewhere in the body where metal ions are deposited [11]. The interaction of memory cells with antigen forms the basis of the Lymphocyte Stimulation Test (LST). The test has previously been used for the diagnosis of allergy to drugs, formaldehyde, epoxides and isothiazolinones [12–14]. MELISA® (Memory Lymphocyte Immuno Stimulation Assay), a further development of the test, measures immunological sensitization induced by mercury, gold, palladium and other metals [15–17]. In this article, cellular responses to metals in various groups of fatigued patients with or without autoimmunity were compared to those of healthy controls. The results indicate significantly increased lymphocytic reactivity to nickel and mercury in fatigued patients compared to healthy subjects.

Material and methods

Patients and controls

Three groups of patients participated in this study. Group A consisted of two subgroups. The first group included 10 fatigued female patients with autoimmune thyroiditis and APS II-III (mean age of 50.2 years). Laboratory findings indicated the presence of autoantibodies against adrenals, ovaries, testes, islets of Langerhans, etc. In the second subgroup there were 12 fatigued patients with autoimmune thyroiditis only (two men and 10 women, mean age of 49.3 years). Since preliminary data did not reveal any differences in the lymphocyte reactivity of the subgroups, the patient data were combined (22 patients, mean age of 49.7 years).

Group B comprised of 28 fatigued patients free of an endocrinopathy referred by the Dept. of Allergology at Na Homolce Hospital in Prague or by their dentists. The patients complained of local and systemic symptoms associated with metallic dental alloys. The following systemic symptoms were common: chronic fatigue, malaise, nausea or vomiting, headache, excessive sweating, dizziness, respiratory disorders, and cardiovascular symptoms such as arrhythmia. Local symptoms were burning or itching of the oral mucosa adjacent to metal restorations, blister eruption, erosion, skin pigmentation, and excessive salivation. The patients were 8 men and 20 women, with a mean age of 45.7 years.

Group C included 22 professional workers without evidence of autoimmunity with a mean age of 52.6 years (range 38 to 57 years). The duration of exposure was more than 15 years. Sixteen were dentists, dental assistants, and technicians. Two professionals, aged 45 and 52 years, worked as grinders in the metal industry and the other two females (37 years and 67 years old) were working as an artist and biochemist, respectively. They reported diffuse symptoms such as tiredness, lack of concentration, and pain in the back, among other symptoms.

The control group (D) consisted of a population sample of 13 individuals, four men and 10 women (mean age of 41.7 years). These subjects were

healthy, without fatigue, and without any abnormal laboratory findings.

The study was approved by the Ethical Commission of the Institute of Endocrinology in Prague. The patients and controls were informed of the study protocol and gave their informed consent.

MELISA®

The blood was collected into six vacutainer tubes with polystyrene beads (Becton Dickinson, UK), defibrinated by shaking and diluted 1:1 using a modified culture medium (RPMI 1640 with Hepes - Gibco BRL - and gentamycin - Krka, Yugoslavia). Mononuclear cells were separated by centrifugation for 30 minutes at 600 g on a Ficoll-Paque gradient (Pharmacia, Uppsala, Sweden), followed by washing. Cells were resuspended in 20% autologous inactivated serum and incubated for 30 minutes at 37°C and in 5% CO2 atmosphere in culture flasks (Costar, USA) for partial depletion of monocytes. After incubation, cells were counted and diluted with complete RPMI 1640 medium with 10% inactivated autologous serum in a concentration of 1 x 10^6 cells per ml. The cells were cultured in 48-well tissue plates (Costar, USA) with prepared antigens. Stock solutions were prepared by dilution of metal salts with filtered tissue water (TW) and were further diluted to working solutions at the time of plate preparation. The details were given previously [15]. As negative controls, wells with 100 μl TW without metal antigen were used. As positive controls served mitogens from Phytolacca Americana (PWM, Sigma concentration 1:50), Concanavallin A (Con A, Sigma; concentration 1:10) and phytohemagglutinine (PHA, Sigma; concentration 1:10). After 5 days, 600 μl of cell suspension from each well was transferred to a new plate and 111 kBq of methyl – ^{3}H thymidine (ÚVVVR, Prague) was added. After 4-hour incubation, the samples were harvested using an automatic cell harvester (Inotech, Switzerland) and counted on a Microbeta counter (LKB/Wallac, Finland). The incorporation of radiolabeled thymidine in metal cultures (counts per minute, cpm) as compared control cultures is expressed as stimulation index (SI):

SI=cpm in experimental cultures/mean cpm in control cultures.

Stimulation index of 2 was regarded as a positive response. Statistical analysis of data was performed using two-tailed Fisher's exact test.

Results

The lymphocyte responses to various metals in patients and controls are shown in Fig. 1 and Table 1. Lymphocytes from patients with autoim-

Table 1. Lymphocyte responses to metals in patients and controls metals

Group / Metal	Fatigued with autoimmune thyroiditis			Fatigued without endocrinopathy			Fatigued professionals			Healthy subjects	
Ag	15.8[1]	(19)[2]	n.s.[3]	24.0	(25)	n.s.	6.7	(15)	n.s.	0.0	(11)
Au	27.8	(18)	n.s.	21.7	(23)	n.s.	22.2	(18)	n.s.	0.0	(12)
Cd	28.6	(14)	n.s.	42.9	(21)	n.s.	16.7	(18)	n.s.	18.2	(11)
Co	11.8	(17)	n.s.	15.8	(19)	n.s.	10.0	(20)	n.s.	0.0	(10)
Cr	6.3	(16)	n.s.	20.0	(20)	n.s.	18.2	(22)	n.s.	0.0	(10)
Cu	6.7	(15)	n.s.	5.6	(18)	n.s.	0.0	(8)	n.s.	0.0	(8)
EtHg	25.0	(8)	n.s.	18.2	(11)	n.s.	17.6	(17)	n.s.	0.0	(7)
Hg	72,7	(22)	0.0008	70,4	(27)	0.0004	50,0	(22)	0.024	8.3	(12)
MeHg	33.3	(9)	n.s.	27.3	(11)	n.s.	21.1	(19)	n.s.	0.0	(6)
Ni	61,1	(18)	0.002	50,0	(24)	0.006	38,1	(21)	0.032	0.0	(10)
Pb	42.9	(14)	n.s.	25.0	(20)	n.s.	21.4	(14)	n.s.	33.3	(9)
Pd	23.1	(13)	n.s.	17.4	(23)	n.s.	26.7	(15)	n.s.	8.3	(12)
PhHg	31.6	(19)	n.s.	20.0	(25)	n.s.	35,0	(20)	0.033	0.0	(11)
Pt	36.4	(11)	n.s.	12.5	(16)	n.s.	40.0	(10)	n.s.	0.0	(9)
Sn	20.0	(15)	n.s.	27.8	(18)	n.s.	7.1	(14)	n.s.	0.0	(9)
Thiomersal	17.6	(17)	n.s.	11.8	(17)	n.s.	0.0	(18)	n.s.	0.0	(9)
Ti	33.3	(12)	n.s.	40.0	(15)	n.s.	11.8	(17)	n.s.	0.0	(7)

[1] **Positive responses** (%); [2] **Number of tests** (in parentheses)
[3] **Statistical significance /p-value** (n.s. = non significant) of the difference in positive response between the particular group A, B, C and healthy subjects (D).

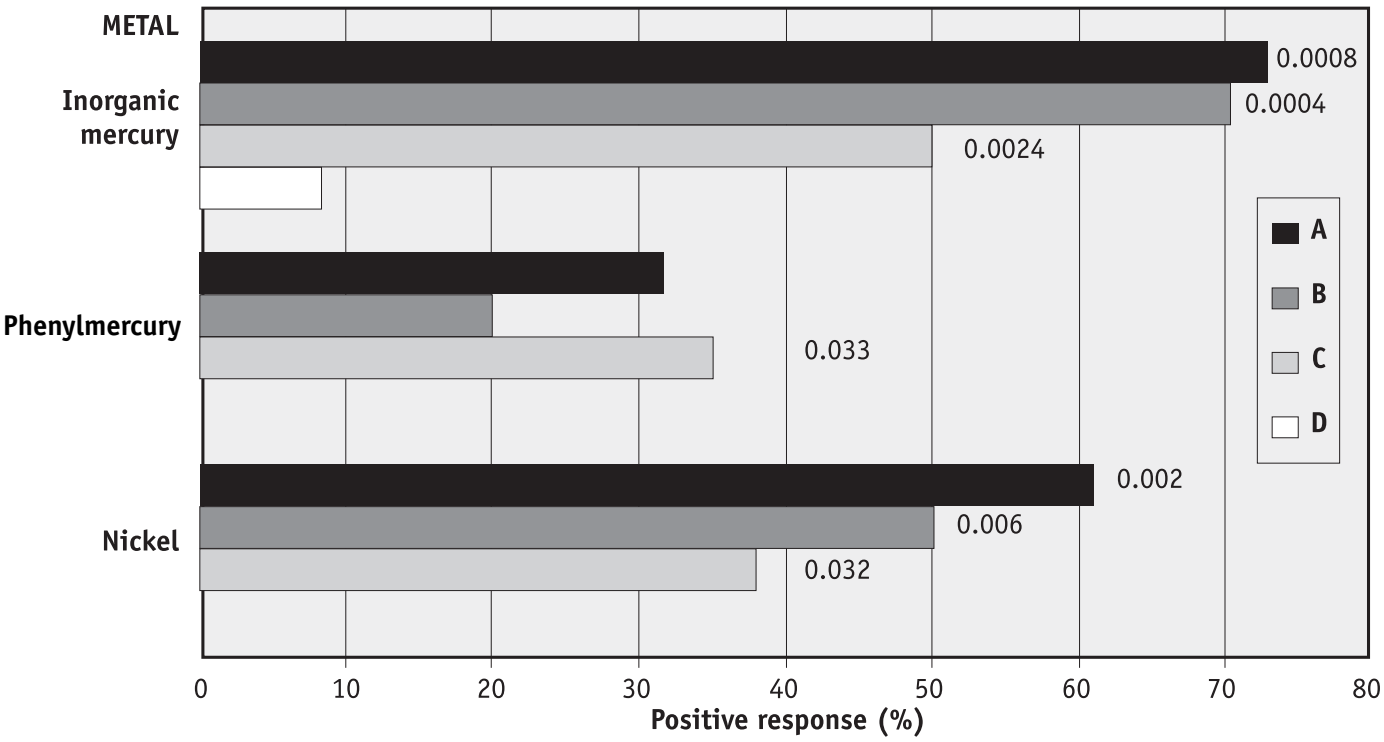

Fig. 1. Number of positive responses to inorganic mercury, nickel and phenylmercury. The numbers indicate p-values of statistically significant differences versus controls.

A - Fatigued patients with autoimmune thyroiditis
B - Fatigued patients without endocrinopathy
C – Fatigued professionals
D – Healthy subjects

Table 2. Lymphocyte proliferation to various metals in case 1.

Antigen in culture	MELISA 1	MELISA 2 (after 6 months)
Inorganic Hg	8.5[1]	1.9
Phenylmercury	3.6	1,0
Thiomersal	2.0	0.5
Nickel	2,4	2.9
Gold	3.3	1.2

[1] **Stimulation Index**

Table 3. Lymphocyte proliferation to various metals in case 2.

Antigen in culture	MELISA 1	MELISA 2 (after 6 months)
Inorganic Hg	14.5[1]	6.0
Phenylmercury	6.0	1.0
Thiomersal	0.2	5.1
Palladium	2.8	0.9
Cadmium	11.5	3.2
Nickel	54.3	6.8
Copper	2.6	0.7

[1]**Stimulation Index**

mune thyroiditis as well as from fatigued patients without endocrinopathies and in fatigued professionally exposed subjects reacted more significantly to inorganic mercury and nickel as compared to healthy controls. In addition, increased lymphocyte reactivity to phenylmercury in fatigued professionals was also observed. The positive lymphocyte responses to other metals did not statistically differ from the healthy controls. In the control group, four individuals showed positive lymphocyte responses, primarily to cadmium and lead.

To examine the possible clinical relevance of the *in vitro* findings, the effect of dental metal replacement on patients' health and lymphocyte reactivity was further studied.

The lymphocyte responses of two representative patients out of a cohort of 10 similarly treated patients is shown in Tables 2 and 3.

A 51-year-old woman (case 1) with autoimmune thyroiditis and rheumatoid arthritis worked as a surgical assistant in a hospital. She was a heavy smoker and was referred to the Institute of Endocrinology due to debilitating fatigue that prevented her from working. Laboratory findings were positive titers of anti-Epstein Barr virus (EBV) and anti-Cytomegalo-

virus IgG antibodies. Dental examinations revealed the presence of amalgam fillings only. The patient's lymphocytes reacted to inorganic mercury, phenylmercury, gold, nickel and thiomersal. Since the patient considered amalgam as a contributing factor to her ill health, amalgam replacement with composites was performed. At the follow-up examination six months later, lymphocyte reactivity to mercury had decreased while the response to nickel was not affected (Table 2). The patient reported improved health two months after amalgam replacement; the arthritic pain disappeared and the level of fatigue decreased significantly. She began working again and her health improvement still continues 18 months later.

A 24-year-old female dental technician (case 2) reported chronic fatigue and allergy to nickel as confirmed by a patch test. The only laboratory findings were positive IgG titers against EBV. The patient was a non-smoker and had dental amalgam fillings only. She complained of debilitating fatigue, which impaired her capacity to work. (Lymphocyte responses to metals are shown in Table 3.) On the basis of *in vitro* results as well as the evidence of clinical metal sensitivity, the patient underwent replacement of dental amalgam with metal-free restorations. The health and *in vitro* lymphocyte reactivity was evaluated six months later. Following amalgam removal, allergic contact dermatitis disappeared and the patient reported significant decrease of fatigue levels. To avoid further exposure to metals, the patient decided to cease working in the dentistry field. Lymphocyte responses to inorganic mercury, cadmium and nickel decreased significantly (Table 3). In contrast, lymphocyte response to thiomersal increased – probably due to a vaccination with thimerosal-containing vaccine prior to the follow-up testing.

Discussion

The results of this study indicate significantly increased lymphocyte reactivity to inorganic mercury and nickel in patients with chronic fatigue syndrome and autoimmune thyroiditis as compared to healthy controls. The similar results were obtained in fatigued patients without endocrinopathies and in fatigued professionals with diffuse symptoms.

The major source of inorganic mercury is dental amalgam, as previously reported by Skare [18]. Regarding the effect of mercury on thyroid function, Kawada and coworkers [19] demonstrated 50% inhibition of Na+Ka+ATPase in the membranous preparations from hog thyroid by mercuric chloride in 10^{-7} M concentration. A significant reduction in *de novo* synthesis of iodothyronines was demonstrated following an intraperitoneal injection of mercury into mice, thus suggesting that mercury may cause a coupling defect in the synthesis of iodothyronines. An interesting finding is also denaturation of hog thyroglobulin in the presence of 8×10^{-3} M mercuric chloride suggesting that thyroglobulin may carry a large binding capacity against mercurials. Barregård and coworkers [20] studied functional impairments of thyroid in occupationally exposed workers. The results indicated inhibitory effects of mercury vapor on 5'- deiodinases which are responsible for the conversion of T4 to the active hormone T3.

Nickel is an ubiquitous metal and the re-exposure is difficult to avoid by sensitized individuals. Most of our patients were females and the increased prevalence of metal sensitivity may be due to the use of nickel-containing earrings. High-quality stainless steel in cooking utensils may release enough nickel to provoke dermatitis in sensitized subjects [21]. Significant amounts may be released by sweat or household detergents [22]. This release is accelerated by an acid pH and thus, in the handling and cooking of acid fruit and vegetables, nickel may be released [23]. Another source of exposure is cigarette smoke. Last but not least, nickel may be found as an impurity in amalgam or as a component of dental crowns and bridges [24 and unpublished observations].

The prevalence of metal sensitivity in patients with CFS and other disorders with unclear etiology has not been studied to a greater extent. Swedish researchers have reported on the increased prevalence of nickel-positive patch tests in patients with fibromyalgia and CFS [25]. Further, in 397 patients referred for metal patch testing, over 50% of the patients exhibited systemic symptoms such as fatigue and muscular pain while the local oral symptoms were less frequent [26]. The patch test data confirmed the results of increased lymphocyte responsiveness to metals *in vitro* in this patient category [15–17].

Positive lymphocyte responses were clinically relevant since patients often reported intolerance to metal earrings and other metallic devices such as jeans buttons, watches and intrauterine devices. Some of them also reported worsening of fatigue and other symptoms following a visit to the dentist. Two patients with clinical and *in vitro* metal reactivity underwent the replacement of amalgam fillings. Both patients reported significant improvement of health as compared to the time prior to amalgam removal. Parallelly, the lymphocyte responses to metals decreased as well. Similar results were

observed in 10 other patients participating in an ongoing long-term study and in a recently reported Swedish study [27].

The improved health observed in many patients following amalgam replacement has been observed by others [28–30]. In a recent study of 6,744 consecutive patients from 34 dental offices in Germany, a higher number of symptoms as well as higher intensity of symptoms were found in patients before amalgam removal compared to the remaining patients [31]. There was no correlation between the intensity of complaints and the number of amalgam-fillings. These findings sugges an immunological rather than a toxicological basis of amalgam-induced side-effects.

The fatigue and total "burn out" in dentists in numerous countries has been previously published [32, 33]. In 1976 Cheraskin [34] observed an increased prevalence of fatigue in dentists with low intake of vitamin C. An analysis of the data on vitamin C intake and fatigue in 411 dentists and their wives revealed a negative relationship.

It is generally recognized that immune activation resulting in cytokine release may cause severe psychosomatic symptoms [35, 36]. Since chronic immune activation could be a plausible explanation of CFS and other alleged psychosomatic disorders, the demonstration of immune activation in CFS patients generated considerable interest in the scientific community. Thus, Buchwald and coworkers reported the increased serum markers of inflammation and immune activation in patients with CFS and chronic fatigue, compared to controls [37]. In a recent review article, Komaroff and Buchwald emphasized that CFS is a definite entity characterized by chronic activation of the immune system, abnormalities of the HPA-axis, and abnormal changes in the brain [38]. The pathological MRI findings in multi-symptomatic patients exhibiting lymphocytic sensitization to metals and disregulation of phenotypic lymphocyte markers have been reported previously [39].

The chronic exposure to corrosion products of dental metals or to metals generally could serve as a trigger of chronic inflammation in sensitized individuals. The disregulation of HPA-axis in CFS has been described by Demitrack et al. in 1991 [40] and in autoimmune thyroiditis by Sterzl and coworkers [41]. The symptoms of fatigue are frequent in other autoimmune disorders, for instance in multiple sclerosis [42].

Allelic components of the major histocompatibility complex (MHC) are associated with various immunological diseases, including allergies to heavy metals. In man, hypersensitivity to heavy metals is more frequent in patients with HLA DR4 antigens [43, 44], and monozygous twins with CFS display an identical pattern of metal sensitization [15]. Hence, in agreement with the animal findings [3], sensitization to metals in man seems to be determined by an individual genetic predisposition.

The introduction of the new method MELISA® is a major step in the improvement of the diagnosis of hypersensitivity to heavy metals, thus allowing the identification of the patients where simple removal of metal fillings will markedly enhance the quality of life.

Acknowledgments

The project was supported by grants 3419-3 and 3472-3 awarded by the Internal Grant Agency of the Ministry of Health of the Czech Republic and by AB Astra, Södertälje, Sweden.

Currently following laboratories perform MELISA®:

- Medilab, Stockholm, Sweden: www.medilab.se;
- Labor Schiwara, Bremen, Germany: www.medlab-schiwara.de
- Institut für Umweltkrankheiten im Kurpark 1D-34308, Bad Emstal, Germany: www.ifu.org
- Laboratoire Ategis, Rue des Chevaux 67, 1640 Rhode - St Genese, Belgium.

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*Neuroendocrinology Letters 1999; **20**:221–228*