Removal of dental amalgam decreases anti-TPO and anti-Tg autoantibodies in patients with autoimmune thyroiditis

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Abstract

OBJECTIVES: The impact of dental amalgam removal on the levels of anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies was studied in patients with autoimmune thyroiditis (AT) with and without mercury allergy.
METHODS: Thirty-nine patients with AT were tested by an optimized lymphocyte proliferation test MELISA® for allergy (hypersensitivity) to inorganic mercury. Patients were divided into two groups: Group I (n = 12) with no hypersensitivity to mercury and Group II (n = 27) with hypersensitivity to mercury. Amalgam fillings were removed from the oral cavities of 15 patients with hypersensitivity to mercury (Group IIA) and left in place in the remaining 12 patients (Group IIB). The laboratory markers of AT, anti-TPO and anti-Tg autoantibodies, were determined in all groups at the beginning of the study and six months later.
RESULTS: Compared to levels at the beginning of the study, only patients with mercury hypersensitivity who underwent amalgam replacement (Group IIA) showed a significant decrease in the levels of both anti-Tg (p=0.001) and anti-TPO (p=0.0007) autoantibodies. The levels of autoantibodies in patients with or without mercury hypersensitivity (Group I and Group IIB) who did not replace amalgam did not change.
CONCLUSION: Removal of mercury-containing dental amalgam in patients with mercury hypersensitivity may contribute to successful treatment of autoimmune thyroiditis.

ORIGINAL ARTICLE

Abbreviations & Units

AT	autoimmune thyroiditis
TPO	thyroid peroxidase
Tg	thyroglobulin
HLA	Human Leukocyte Antigen
MELISA®	Memory Lymphocyte Immuno Stimulation Assay
LST	Lymphocyte Stimulation Test
SI	Stimulation Index

Introduction

Endocrine organs can be affected by autoimmune processes leading to organ-specific autoimmune diseases. The thyroid gland is vulnerable to such an attack, resulting in AT. The risk to develop AT is increased predominantly in women with autoantibodies against anti-TPO and anti-Tg [38].

For AT development several factors are necessary. One factor is the genetic predisposition, which is linked mainly to the antigens of the HLA system, namely to HLA B8, B15, B5, DR3, and DR4 [2,10]. Additional factors are environmental, such as viral and bacterial infections [12], especially *Yersinia enterocolitica* [5,6] and *Helicobacter pylori* [9,11], iodine intake [19], and allergy to heavy metals [4, 35, 36].

The human body is constantly exposed to metals and metal-containing compounds. Some metals are crucial in trace amounts for normal metabolic functions, while others have toxic effects. The key factors influencing the harmfulness of metals are cumulative concentration, duration of exposure, and genetic susceptibility. Many harmless metals may become allergens or toxic if administered on a chronic basis [24].

Metal-induced allergy is based on the reaction of the allergen with the surface of memory T-lymphocytes previously sensitized to that specific allergen. 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 [15].

Dental amalgam is the most common source of inorganic mercury for the human body [25] and is considered to be an important risk factor for patients with autoimmune diseases [4, 22, 23, 34]. Regarding the effect of mercury on thyroid function, Kawada and coworkers [16] demonstrated 50% inhibition of Na+Ka+ATPase in the membranous preparations from hog thyroid gland 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. Denaturation of hog Tg in the presence of 8 x 10^{-3} M mercuric chloride suggests that Tg may carry a large binding capacity for mercurials [16]. Barregard and coworkers [3] studied functional impairments of the thyroid gland 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.

The interaction of memory cells with antigen forms the basis of the Lymphocyte Stimulation Test (LST). The test has been used previously to diagnose allergy to drugs, formaldehyde, epoxides and isothiazolinones [30, 31, 33]. MELISA® (Memory Lymphocyte Immuno Stimulation Assay), a modification of the LST, is a valuable tool for detecting patients with hypersensitivity to heavy metals and also for monitoring metal allergies [20, 28, 29, 32, 34, 37].

AT is caused mainly by cytotoxic lymphocytes activated by dendritic cells as well as by Th1 subpopulation of lymphocytes infiltrating thyroid gland. Anti-TPO and anti-Tg autoantibodies are regarded as diagnostic markers of AT [1]. These autoantibodies can also directly activate antibody-dependent cell-mediated cytotoxicity and thus play a role in the destruction of follicular cells [8].

In this paper we report the decrease in autoantibodies against thyroid antigens six months after replacement of amalgam fillings by non-metallic materials.

Materials & Methods

Patients

Patients included in this study were referred to the Institute of Endocrinology, Prague. Diagnosis of AT was based on clinical and ultrasound findings. All patients had auto-antibodies against TPO and/or Tg and were in euthyroid state. At the beginning of the study, patients were examined by a dentist (JP), and dental status was recorded. Thirty-nine patients who had only amalgam restorations were selected for further study. The mean number of amalgam fillings was 9, range 6 to 16. All patients also completed a questionnaire regarding health status and metals exposure in the past.

Patients were divided into two groups according to their responsiveness to inorganic mercury *in vitro* (MELISA® test); Group I consisted of 12 female patients with AT who did not respond to inorganic mercury *in vitro*. Group II consisted of 27 female patients with AT and with positive mercury-specific lymphocyte responses as diagnosed by MELISA® (average response: Stimulation Index [SI] = 8.8; range of response: SI 2.2 to 21.4). In the latter group 15 patients underwent amalgam replacement (Group IIA) and 12 patients did not undergo amalgam replacement (Group IIB).

Methods

Determination of hypersensitivity to mercury

The determination of hypersensitivity to inorganic mercury was performed by the MELISA® test according to standard procedure [32]. In brief, lymphocytes were isolated on Ficoll gradient and cultivated in RPMI 1640 medium with 10% of inactivated human AB+ serum for 5 days with different concentrations of inorganic mercury. Radiolabeled thymidine was added to measure the

lymphocyte proliferation through the uptake into DNA by dividing cells and expressed as Stimulation Index (SI). SI=cpm in mercury-treated cultures/mean cpm in control cultures. SI< 2 is considered a negative response, SI 2–2.9 weekly positive response and SI ≥3 positive response. MELISA® was performed on all patients at the beginning of the study, and a follow-up test was performed six months later.

Determination of antibodies

Autoantibodies against TPO and Tg were determined by ELISA using the kits Aeskulisa A-Tg and Aeskulisa A-TPO (Aesku Diagnostics, Wendelsheim, Germany). The first determination of anti-TPO and anti-Tg autoantibodies was performed in all patients at the beginning of the study, and a follow-up test was performed six months later.

Amalgam removal

Following discussion with the patient and with the patient's informed consent, amalgam was removed in two to four sessions under maximal protection, such as use of rubber dam, powerful sucking appliance and fresh air [14], and replaced by composites and/or ceramic inlays.

The oral examination and the replacement of amalgam by non-metallic materials was performed in The Institute of Dental Research, First Medical Faculty and General Faculty Hospital, Charles University, Prague.

Statistical evaluation

For the evaluation of the differences in the groups, the signed rank test was used. The differences between the groups were evaluated by Kruskall-Wallis ANOVA on ranks together with Kruskal-Wallis multiple comparison tests using Statistica statistical package.

Results

A significant decrease of lymphocyte stimulation by inorganic mercury after amalgam replacement was found by signed rank test in Group IIA only ($p = 0.0013$, Table 1). The other groups did not differ in the responsiveness to inorganic mercury in same period of time.

In parallel, a significant decrease in anti-TPO ($p < 0.001$, Fig. 1) and anti-Tg ($p = 0.001$, Fig. 2) autoantibody levels in the group of patients who removed amalgam fillings were also found. Kruskal-Wallis ANOVA of autoantibody levels in the follow-up test together with the multiple comparison test showed a significant decrease of anti-TPO ($p = 0.010$, Fig. 3) and anti-Tg ($p < 0.001$, Fig. 4) autoantibodies for Group IIA.

Regarding the health status of patients in Group IIA (based on responses in a questionnaire), 70% reported a substantial improvement, 21% reported an improvement, 9% reported unchanged health, and none of patient reported deterioration of health following amalgam replacement.

Discussion

The important role that heavy metals play in the development of many autoimmune diseases is currently well-recognized [22, 23]. Hypersensitivity to metals results in a wide range of clinical and sub-clinical symptoms such as chronic fatigue, depression, sleep disturbances, and others [34, 37]. Patients with these symptoms often report intolerance to metal earrings and other metallic devices such as jeans buttons, watches, and intrauterine devices. Some patients also report worsening of fatigue and other symptoms following a dental visit [27, 34]. Metal-induced inflammation has also been found as a contributing factor in some patients suffering from multiple sclerosis and in other autoimmune diseases as described by us [22, 36] but also by others [17, 18, 27, 34, 39]. This study indicates that amalgam replacement in mercury-sensitive patients results not only in the decrease of mercury-specific lymphocyte reactivity as published previously [27] but that the specific anti-thyroid autoantibodies are down-regulated as well. These findings suggest an immunological rather than toxicological basis of amalgam-induced side-effects in susceptible patients. Unlike patch test, MELISA®, an *in vitro* test, does not carry the risk of local toxic reaction due to application of metallic compounds on the skin or of inducing or exacerbating systemic hypersensitivity.

Table 1: Lymphocyte stimulation by inorganic mercury at the beginning of study and 6 months later as measured by MELISA®.

Group I		Group IIA*		Group IIB	
Before	After	Before	After	Before	After
0.12	0.36	21.44	12.89	4.26	4.81
0.23	0.45	15.76	0.25	4.32	3.25
1.90	1.65	16.1	11.31	10.02	8.89
1.77	1.92	14.45	12.44	2.28	3.67
0.68	0.52	8.59	2.49	14.34	12.49
1.46	1.04	2.15	2.15	4.40	5.33
1.30	1.10	9.54	0.49	2.15	3.62
0.30	0.64	3.55	3.4	15.21	11.61
0.46	0.31	8.53	1.85	6.01	5.64
0.71	0.81	13.2	4.67	5.36	4.13
0.51	0.69	9.21	3.68	4.33	3.69
0.01	0.95	11.81	3.95	7.58	8.11
		12.11	4.81		
		2.58	2.58		
		4.96	0.01		

Lymphocyte stimulation is expressed as maximal stimulation index (SI). **Group I:** AT patients who did not respond to inorganic mercury *in vitro*. **Group IIA:** AT patients with positive lymphocyte responses to mercury *in vitro* who underwent replacement of amalgam fillings. **Group IIB:** AT patients with positive lymphocyte responses to mercury *in vitro* whose amalgam fillings were left in place. **Before**: at the beginning of the study. **After**: six months after first examination or amalgam replacement.
* denotes a significant difference – (signed rank test)

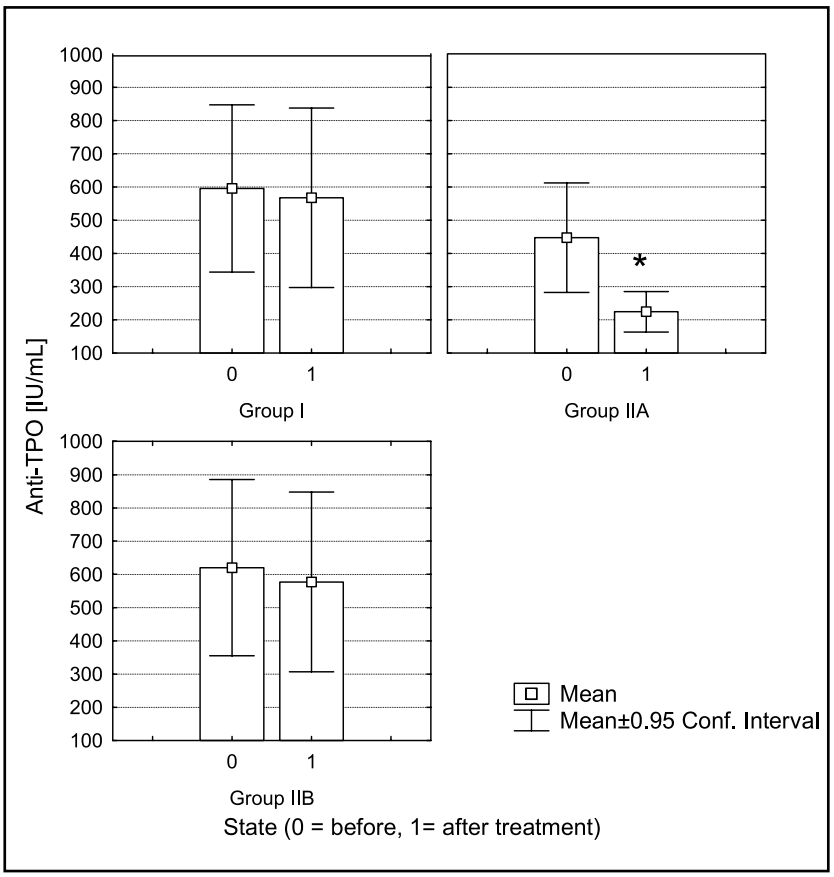

Figure 1: Differences within groups for anti-TPO autoantibodies tested at the beginning of study (0) and six months later (1).
* denotes a significant difference – (signed rank test).
Group I: AT patients who did not respond to inorganic mercury *in vitro.*
Group IIA: AT patients with positive lymphocyte responses to mercury *in vitro* who underwent replacement of amalgam fillings.
Group IIB: AT patients with positive lymphocyte responses to mercury *in vitro* whose amalgam fillings were left in place.

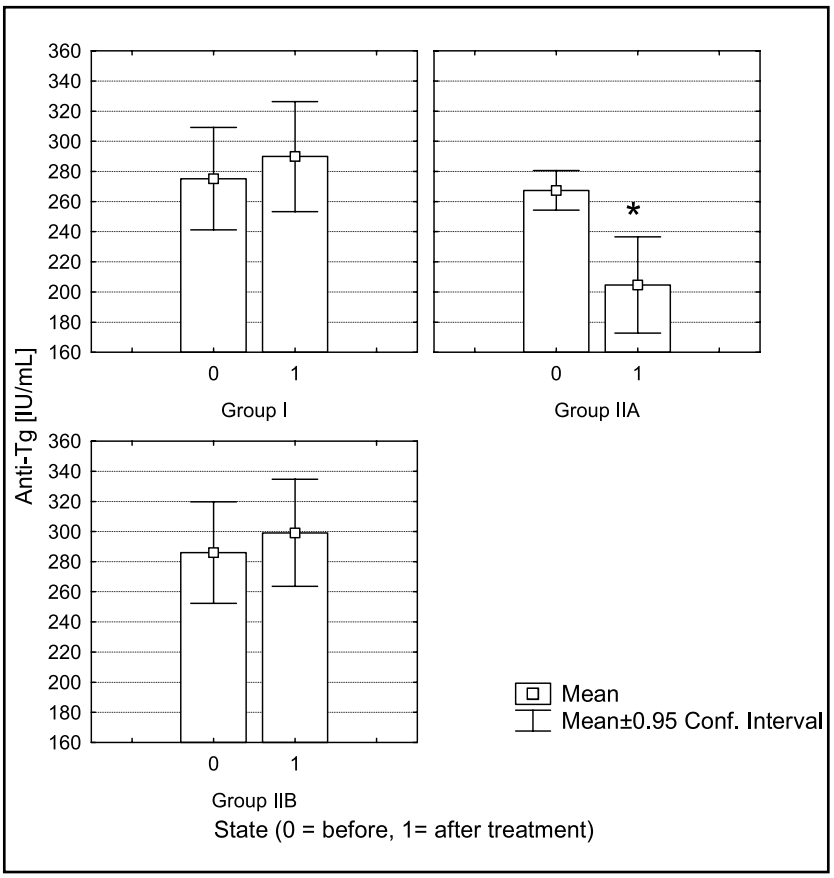

Figure 2: Differences within groups for anti-Tg autoantibodies tested before (0) and six months later (1).
* denotes a significant difference – (signed rank test).
Group I: AT patients who did not respond to inorganic mercury *in vitro.*
Group IIA: AT patients with positive lymphocyte responses to mercury *in vitro* who underwent amalgam replacement.
Group IIB: AT patients with positive lymphocyte responses to mercury *in vitro* whose amalgam fillings were left in place.

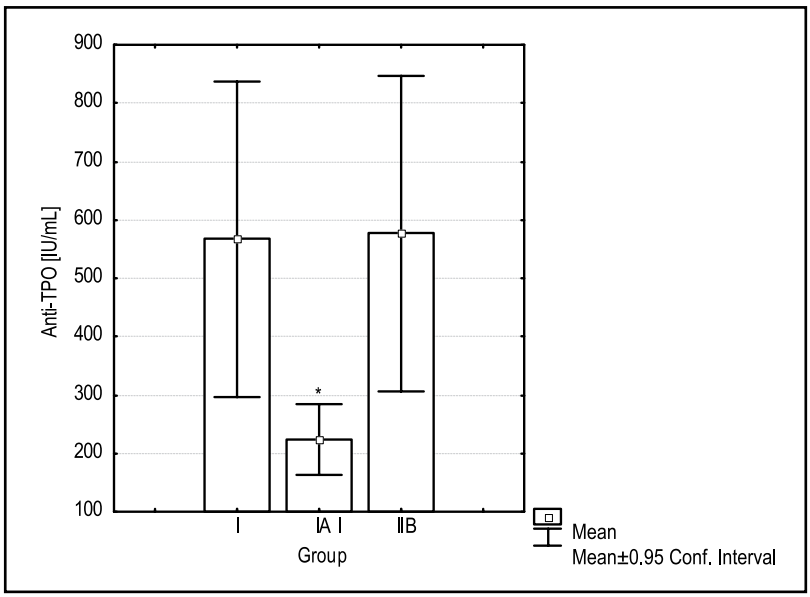

Figure 3: Differences among groups for anti-TPO autoantibodies tested at the end of the study. * denotes a significant difference – (Kruskal-Wallis test).
Group I: AT patients who did not respond to inorganic mercury *in vitro.*
Group IIA: AT patients with positive lymphocyte responses to mercury *in vitro* who underwent amalgam replacement.
Group IIB: AT patients with positive lymphocyte responses to mercury *in vitro* whose amalgam fillings were left in place.

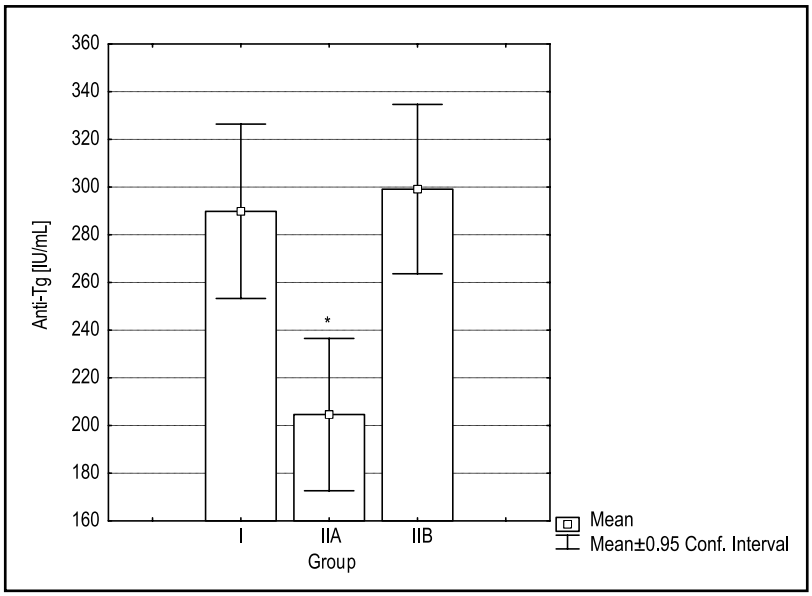

Figure 4: Differences among groups for anti-Tg autoantibodies tested at the end of the study. * denotes a significant difference – (Kruskal-Wallis test).
Group I: AT patients who did not respond to inorganic mercury *in vitro.*
Group IIA: AT patients with positive lymphocyte responses to mercury *in vitro* who underwent amalgam replacement.
Group IIB: AT patients with positive lymphocyte responses to mercury *in vitro* whose amalgam fillings were left in place

Increased lymphocyte reactivity to inorganic mercury and nickel in patients with AT has been reported previously [36].

AT is the most frequent organ-specific autoimmune endocrinopathy. Infiltrating lymphocytes and T cell-mediated cytotoxicity are involved in the pathogenesis of AT [26]. Many researchers stress the significance of autoantibodies as markers of the risk of organ-specific autoimmune diseases; however, these antibodies also play a role in the pathogenesis of these diseases [8]. The decrease of autoantibody levels, especially in systemic autoimmune diseases, can result from altered "environmental" conditions such as the eradication of *Helicobacter pylori* in patients with AT [7]. Similarly, our data show a statistically significant decrease in anti-TPO and anti-Tg autoantibodies in patients with hypersensitivity to inorganic mercury after replacement of dental amalgam fillings. This hypersensitivity may be linked to individual genetic predisposition [21] as described for the development of AT [13].

In conclusion, these data suggest that while heavy metal exposure may not be the general pathogenetic mechanism of AT development, it can be an important co-activating factor in genetically predisposed individuals. The removal of dental amalgam fillings can, under those circumstances, contribute to clinical and laboratory remission of AT.

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