

Screening of metal ions among patients with autoimmune thyroid diseases – a pilot study

Abstract:

Background: Autoimmune thyroid diseases (AITDs) are organ specific disorders of unknown etiology. Metal ions have been implicated in induction of autoimmunity.

Methods: This analytical cross-sectional pilot study of 100 patients with AITDs (73 females and 27 males; mean age of 34.6±14.7 years) and 100 normal healthy individuals (mean age 38.1±9.4 years; 66 females and 34 males) was performed at King Khalid University Hospital, Riyadh. Serum nickel sulfate, titanium and chromium were measured by inductively coupled plasma-mass spectrometry (ICP-MS) instrument (Perkin-Elmer).

Results: Mean serum concentration of nickel sulfate among patients with AITDs (0.0068 ± 0.002 µg/L; 95% CI 0.006330-0.007410) was significantly higher than the normal controls (0.0059 ± 0.001 µg/L; 95% CI 0.005598-0.006288; $p < 0.004$). The mean serum levels of titanium among the patients with AITDs (0.053 ± 0.09 µg/L; 95% CI 0.5195-0.5567) and the control group (0.053 ± 0.09 µg/L; 95% CI 0.5170-0.5538) were no different. Similarly, serum chromium levels among patients with AITDs (0.177 ± 0.02 µg/L; 95% CI 0.1723-0.1825) and controls (0.175 ± 0.02 µg/L; 95% CI 0.1715-0.1813) were also devoid of any statistical significance. High serum level of nickel among the patients with AITDs did not correlate with anti-thyroid antibodies.

Conclusion: Higher serum levels of nickel sulfate among patients with AITDs may be associated with development of thyroid autoimmunity. Large scale studies are recommended to validate the findings of this study.

Key words: Nickel sulfate, titanium, chromium, autoimmune, thyroid diseases.

27 **Introduction:**

28 Auto immune thyroid diseases comprise of Graves' disease and chronic auto-immune thyroiditis
 29 clinically manifesting as hyper and hypothyroidism respectively.¹ The pathological hallmark of thyroid
 30 autoimmunity is the intense lymphocytic infiltration and the presence of anti-thyroglobulin, anti-thyroid
 31 peroxidase and thyroid stimulating hormone receptor antibodies.² Whereas the precise mechanism of
 32 induction of auto-immunity remains obscure repeated exposure to metals has been implicated in immune
 33 mediated pathologies including allergic and autoimmune reactions.³ The capacity of metals to trigger
 34 autoimmune reactions is based upon the ability of some metals to induce MHC-restricted T cell activation
 35 against self-peptides by establishing non-covalent bridging between self-peptide-major histocompatibility
 36 complex (MHC) and T cell receptor.⁴

37
 38 Nickel is a ubiquitous metal and is the principle cause of allergic contact dermatitis along with other less
 39 frequently implicated metals such as cobalt, gold and chromium.⁵ Data linking nickel with autoimmunity
 40 are lacking. Experimental studies however have revealed that subsequent to administration of nickel
 41 either by oral or subcutaneous routes Norway rats develop autoimmunity with the production anti-nuclear
 42 antibodies.⁶ Similarly, data regarding titanium induced inflammation are scarce. There is however one
 43 report describing titanium induced hypersensitivity reaction following placement of an occlusive device
 44 for patent foramen ovale.⁷ The nature of contact, the length and the route of exposure along with the dose
 45 of the nickel or other metal ions may be the main determinants for induction of autoimmunity in a
 46 susceptible individual. This study was performed to assess the blood levels of nickel, titanium and
 47 chromium among patients with AITDs.

48

49 **Patients and methods**

50 This analytical cross-sectional study was performed in the clinical chemistry unit at King Khalid
 51 University Hospital, Riyadh between March 2019 and May 2020. A total of 100 patients with thyroid
 52 autoimmune diseases harboring anti-thyroid antibodies were enrolled in the study. The patients were

included in the study on the basis of presence of anti-thyroid antibodies, ultrasonographic and histological evidence for autoimmune diseases. Details about the presenting signs and symptoms could not be obtained because of lack of access to patient records. This group of patients included 73 females and 27 males with the mean age of 34.6 ± 14.7 years. A group of 100 healthy controls was also recruited from the members of hospital staff and blood bank donors. This group comprised of 66 females and 34 males (mean age 38.1 ± 9.4 years) with no serological evidence of thyroid autoimmunity (Table 1). Since there was no previous report investigating nickel association with AITDs the sample size was chosen arbitrarily to perform this pilot study. Based on the thyroid hormone profiles patients with thyroid autoimmunity were grouped into five categories. Majority of the patients 48 (48%) had low thyroid stimulating hormone (TSH) with normal free thyroxine (fT4) and were categorized as sub-clinical hyperthyroidism. There were 39 (39%) patients with sub-clinical hypothyroidism with high TSH and normal fT4 blood levels. Chronic autoimmune thyroiditis group comprised of 8 (8%) patients with high TSH and low fT4 blood levels. Graves' disease group with high levels of fT4 and low blood levels of TSH included 5 (5%) patients. Euthyroid status with normal levels of TSH and fT4 was found in 43 (43%) AITD patients (Table 2). Anti-thyroid antibodies both anti-thyroid peroxidase (TPO) and anti-thyroglobulin (TG) were assessed by enzyme-linked immunosorbent assay (ELISA) using Quanta Lite kits (Inova diagnostics, Ingbert, Germany). For the interpretation of results cut off value for anti-TPO antibodies was 100 WHO units and for anti-TG antibodies was 60 WHO units. Thyroid hormones were assessed by electrochemiluminescence assay using Cobas e 411 analyzer (Roche diagnostics, Mannheim, Germany). The normal range for TSH was 0.25 to 5mIU/L and 12 to 22pmol/L for fT4. After obtaining informed consent 5 ml of venous blood was collected both from patients and controls in a plain vacutainer. Each sample was allowed to clot and serum was extracted after centrifugation. Serum concentrations of nickel, titanium and chromium were measured by inductively coupled plasma-mass spectrometry (ICP-MS) instrument (Perkin-Elmer). This study was approved by the institutional review board of the College of Medicine.

Statistical analysis:

Data were analyzed by MedCalc computer software version 14.8.1. Normally distributed numeric data were summarized as mean \pm standard deviation, non-normally distributed data were expressed as median and interquartile range and categorical data were summarized as numbers and percentages. Comparison between the groups was performed using *t* test and a $p < 0.05$ was considered statistically significant.

Results:

Fig. 1 describes comparison of serum levels of nickel sulfate between patients with AITDs and normal controls. The mean serum level of nickel sulfate among patients with AITDs was 0.0068 ± 0.002 $\mu\text{g/L}$ (95% CI 0.006330 to 0.007410) whereas among the controls the mean serum level of nickel sulfate was 0.0059 ± 0.001 $\mu\text{g/L}$ (95% CI 0.005598 to 0.006288). Comparative analysis revealed that patients with AITDs harbored significantly higher levels of nickel sulfate in their blood compared to the normal healthy controls ($p < 0.004$). The mean serum levels of titanium among the patients with AITDs (0.053 ± 0.09 $\mu\text{g/L}$; 95% CI 0.0195 to 0.5567) and the normal healthy controls (0.053 ± 0.09 $\mu\text{g/L}$; 95% CI 0.5170 to 0.5538) were no different (Fig. 2). Similarly, serum level of chromium among the patients with AITDs was 0.177 ± 0.02 $\mu\text{g/L}$ (95% CI 0.1723 to 0.1825) and among the healthy controls was 0.175 ± 0.02 $\mu\text{g/L}$ (95% CI 0.1715 to 0.1813). The difference between the two groups was devoid of statistical significance (Fig. 3). In order to assess if the elevated serum levels of nickel sulfate had any relationship with the concentration of anti-thyroid antibodies, correlations were sought between serum nickel and anti-TPO and anti-TG antibody concentrations. The serum concentration of nickel sulfate did not correlate either with anti-TPO or anti-TG antibodies.

Discussion:

Among the metal ions investigated in the present study blood level of nickel sulfate was significantly higher among patients with AITDs. Data linking nickel with development of AITDs are lacking. However, metals as haptens have been implicated in various immune processes and inflammation.⁸ Systemic nickel allergy syndrome (SNAS) has recently been described as a chronic inflammatory disorder and has been categorized as a distinct entity.⁹ It occurs in about 20% of patients with nickel allergy and is characterized by eczema, systemic contact dermatitis along with other manifestations involving gastrointestinal, neurological and respiratory systems.¹⁰ Although the exact mechanism of inflammation in SNAS remains obscure a mixed Th1 and Th2 immune responses have been described in the pathogenesis of SNAS.¹¹ It has recently been observed that chronic autoimmune thyroiditis tends to occur two folds more frequently among patients with SNAS compare to occurrence of chronic autoimmune thyroiditis among patients suffering from other autoimmune disorders.¹² It is therefore quite conceivable that nickel plays a pivotal role in SNAS and may also be involved in induction of thyroid autoimmunity by enhancing thyroid antigenicity and promoting autoimmune responses.

Nickel sulfate is widely distributed in the environment and human exposure may occur by inhalation in electroplating industry¹³, ingestion of vegetables, fruits, and cereal crops grown in contaminated areas¹⁴, physical contact in the form of wearing nickel earrings, piercing jewelry¹⁵ keys¹⁶ laptop computers¹⁷, children toys¹⁸ and release from nickel containing dental restorative materials.¹⁹ After gaining access into human body nickel binds to serum albumin forming a human serum albumin-nickel complex in the vicinity of contact between T cell receptor and antigen presenting cell exposed MHC molecules thus facilitating nickel delivery.²⁰ Binding of nickel may alter self-antigens that may trigger presentation of MHC embedded self-peptide resulting in activation T lymphocytes against self-peptides and induction of autoimmunity.²¹ Existence of nickel specific T cells have already been described among patients with contact allergy to nickel²² and it is possible that this subset of T lymphocytes may be involved in

autoimmune responses. In addition, one of the most debilitating nickel induced affliction is induction of apoptosis²³ that may also be critical for tissue damage associated with AITDs. Nickel along with other metals such as mercury and gold elicits delayed type of hypersensitivity reaction and has been implicated in the pathogenesis of rheumatoid arthritis, systemic lupus erythematosus and Sjögren's syndrome²⁴ suggesting a possible role of the metal ions in induction of systemic autoimmunity.

Serum levels of titanium and chromium between the patients with AITDs and controls were no different in this study. Insertion of nickel–titanium chin implant for cosmetic reasons in a female has been shown to induce systemic autoimmune syndrome mimicking clinical features of systemic lupus erythematosus Kikuchi–Fujimoto syndrome, adult onset Still's disease, antiphospholipid syndrome, and hemophagocytic syndrome.²⁵ It is possible that exposure to nickel and titanium in conjunction may have enhanced the antigenic nature of self-antigens to trigger autoimmune responses. In addition, an experimental study investigating long-term exposure to titanium in mice has demonstrated that titanium dioxide induced inflammatory responses are mediated by activation of NFκB.²⁶ Similarly, it has been observed among patients undergoing fixed orthodontic treatment that increasing concentrations of nickel and chromium in gingival crevicular fluid are associated with severity of inflammatory changes in the gingival tissues.²⁷ Despite no elevation in titanium and chromium levels these metals as haptens may contribute in inflammatory responses in conjunction with higher concentration of nickel ions. Metal ions by altering the antigenic characteristics of self-peptides along with their potential to induce cytokines may be involved in induction of autoimmunity. AITDs are single organ autoimmune diseases and it is difficult to exclude the presence of local predisposition in terms of genetic factors in the pathogenesis of AITDs.

In conclusion, patients with AITDs were found to have significantly higher concentration of serum nickel sulfate suggesting an association between nickel and thyroid autoimmunity. Data describing role of nickel sulfate in the induction of autoimmune diseases are scarce and the findings of this study require validation in large scale studies. Furthermore, there appears to be a need of investigation for identification of local

predisposing factors in the thyroid tissue among susceptible individuals for induction of thyroid autoimmunity.

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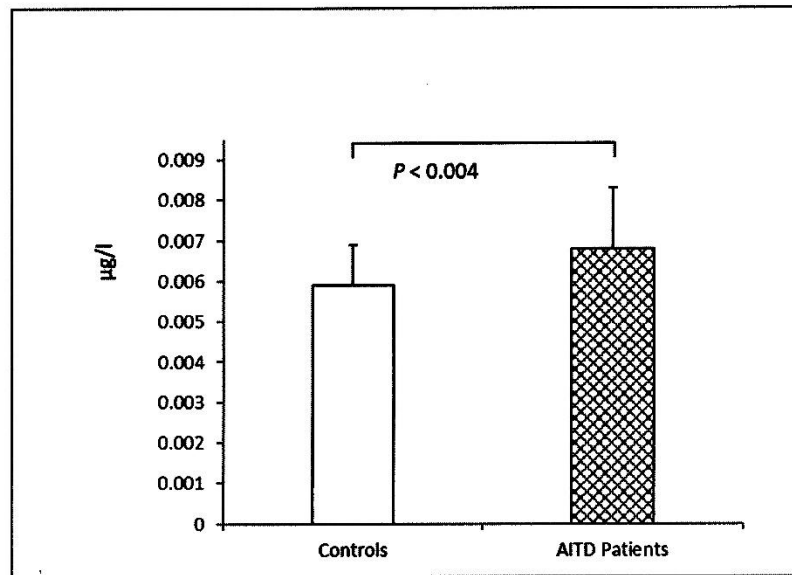
Legends for figures:

Fig 1. Comparison of serum levels of nickel sulfate between patients with autoimmune thyroid diseases and normal healthy controls.

Fig 2. Comparison of serum levels of titanium between patients with autoimmune thyroid diseases and normal healthy controls.

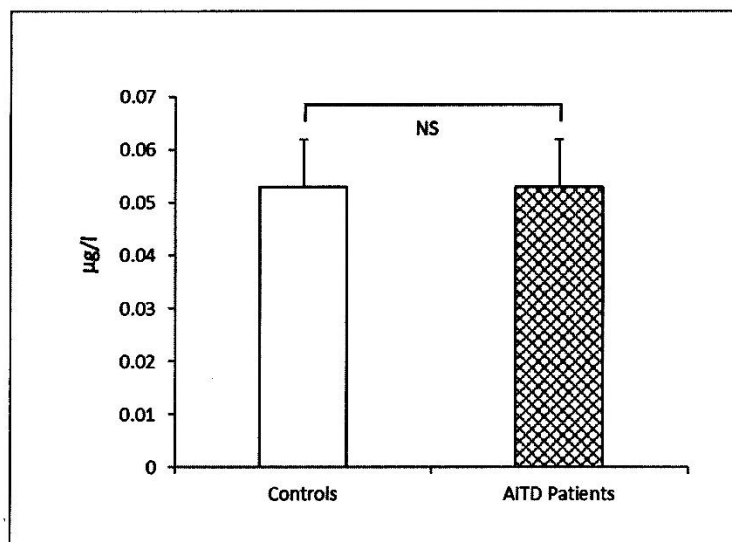
Fig 3. Comparison of serum levels of chromium between patients with autoimmune thyroid diseases and healthy controls.

Fig 1. Comparison of serum levels of nickel sulfate between patients with autoimmune thyroid diseases and normal healthy controls



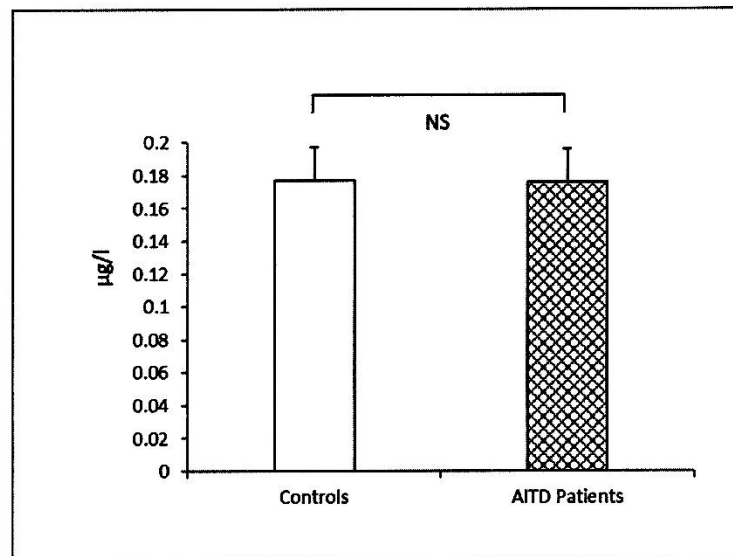
Controls: n = 100, Autoimmune thyroid disease (AITD) patients: n = 100

Fig 2. Comparison of serum levels of titanium between patients with autoimmune thyroid diseases and normal healthy controls



Controls: n = 100, Autoimmune thyroid disease (AITD) patients: n = 100, NS = Not significant

Fig 3. Comparison of serum levels of chromium between patients with autoimmune thyroid diseases and normal healthy controls



Controls: n = 100, Autoimmune thyroid disease (AITD) patients: n = 100, NS = Not significant

Table 1. Characteristic features of patients with autoimmune thyroid disease and normal healthy controls.

Variable	Patients	Controls
Number	100	100
Males n (%)	27 (27%)	34 (34%)
Females	73 (73%)	66 (66%)
Mean age \pm sd years	34.6 \pm 14.7	38.1 \pm 9.4
TG antibodies	67%	ND
TPO antibodies	93%	ND
Coexisting TG and TPO	55%	-

TPO = Thyroid peroxidase, TG = Thyroglobulin, ND = Not detected, sd = Standard deviation

Table2. Categories of patients with autoimmune thyroid diseases based upon thyroid hormone profile and anti-thyroid antibodies.

Condition	n (%)	TSH		fT4		TG Abs		TPO Abs	
		NR	0.25-5	NR	12-22	>60	WHO	>100	WHO
		mIU/L		pmol/L		Units		Units	
GD	5 (5%)	0.05 (0.045)		28.14 (34.96)		154.2 (383.3)		218.8 (1370)	
SH	5 (5%)	0.14 (0.265)		21.4 (8.42)		141.7 (476.3)		795.7 (732.5)	
CAT	8 (8%)	26.93 (97.62)		10.68 (7.19)		734.9 (1133.5)		1593.5 (1788.5)	
SAH	39 (39%)	9.13 (8.62)		15 (3.5)		164 (433.12)		756.42 (1298.3)	
ET	43 (43%)	2.27 (1.14)		15.5 (3.86)		218.5 (714.3)		536.9 (1026)	

Each value is represented as median and interquartile range, GD = Graves' disease, SH = Subclinical hyperthyroidism, CAT = Chronic autoimmune thyroiditis, SAH = Subclinical autoimmune hypothyroidism, ET = Euthyroid. TG Abs = Thyroglobulin antibodies, TPO Abs = Thyroid peroxidase antibody, TSH = Thyroid stimulating hormone, fT4 = Free T4, n = Number, NR = Normal range

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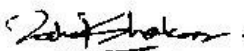
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