



# An ICP-MS Study for Quantitation of Nickel and Other Inorganic Elements in Urine Samples: Correlation of Patch Test Results with Lifestyle Habits

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Received: 2 December 2020 / Accepted: 9 February 2021

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## Abstract

Nickel (Ni) is a frequently used metal in the production of many products, and Ni exposure occurs in humans through food, inhalation, and skin. Studies reported Ni as the most common allergen diagnosed in the serial patch test. The main purpose of our study was to determine the concentration of Ni and other elements in the urine samples of individuals with Ni-sensitivity and to determine the effect of lifestyle habits on these variables. This study was conducted with 388 participants; additionally, both Ni patch test and inorganic analysis (for 24 elements) method in urine with inductively coupled plasma-mass spectrometry (ICP-MS) were applied to all participants. Ninety-two (23.7%) of the participants were found to have nickel sensitivity with a mean urine concentration of 4.475 µg/g creatinine (median=4.260; SD=2.527). The urine nickel concentration was significantly different between female and male participants ( $p = 0.001$ ). We found a significant correlation between Ni and other metals alongside various element pairs. The mean urine Ni concentration of the individuals with a positive Ni sensitivity test result was not different from that of the participants without Ni sensitivity (4.475 vs 4.256 µg/g creatinine, respectively;  $p = 0.068$ ). The high accuracy, recovery, and repeatability data obtained from the analyses indicate that this study is efficient and appropriate for the quantitative determination of nickel and other elements in urine samples. Ni-containing objects and Ni-rich foods should be of concern for individuals with hypersensitivity reactions to the element.

**Keywords** Nickel · Contact dermatitis · Occupational exposure · Imitation jewelry · ICP-MS

## Highlights

- Nickel is a metal with a main source of exposure in water, foods, and daily equipment.
- Occupational exposure to nickel is frequent.
- The mean urine nickel concentration of the female participants was significantly higher than males', possibly as a result of their daily habitual exposure to cosmetic products and imitation jewelry.
- The nickel concentration in the urine increased with an occupational contact.
- The nickel concentrations in the urine samples of the participants who used penetrating jewelry such as earrings and piercings were significantly higher.

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## Introduction

Nickel (Ni) is a silvery-white metal that is generally used as a base metal in the production of stainless steel, but it is also used in the production of electrical equipment, jewelry, household appliances, and coins [1].

Ni exposure occurs in humans through food, inhalation, and skin. Although the target organ of Ni is the respiratory system, it can lead to chromosome breaks at the molecular level [2]. Toxic effects of Ni depend on the type of the compound, physical form, concentration, and the route of exposure. The main source of exposure to Ni is water and foods, as 0.01–0.1 mg/kg with large variations depending on the Ni content of the consumed food and less than 0.025 mg Ni/day for the drinking water and 1–3 ng/m<sup>3</sup> for the ambient air. On the other hand, exposure level to Ni by inhalation is below 0.03 µg/m<sup>3</sup> for a non-smoker who resides in an urban area, whereas the exposure rate increases up to 124 µg/day with smoking (40 cigarettes/day) [3, 4].

A variety of Turkish studies with large sample sizes reported that the most common allergen diagnosed in the standard

serial patch test was Ni, and its sulfate compound was the most frequent sensitized form [5, 6]. A national database study reported that 17.4–31.9% of women and 3.1–10.8% of men are allergic to Ni in the European population [7]. The corrosive power of human sweat allows the metal in an object such as watch, earring, and coin which is in frequent contact with the organism to diffuse into the skin. The exposure level depends on the Ni content of the object and results in aggregation on the skin surface and absorption through the skin. Prolonged exposure to Ni and its compounds through the skin causes irritation of the skin and may develop Ni sensitivity which can further progress to allergic contact dermatitis [8]. Also, The Turkish Dermatology Association, Dermatoallergy Study Group reported that excessive consumption of some vegetables and fruits, nuts, legumes, ready-to-eat foods, meat products which are Ni sources should be avoided in order to prevent a Ni sensitivity [9].

Also, a recent scientific opinion by the European Food Safety Authority (EFSA) reported that these food categories are the main contributors of lower bound (LB) chronic dietary exposure, ranging 31–49%, and the highest estimated chronic dietary exposure to nickel was in toddlers. On the other hand, beans, coffee, ready-to-eat soups, chocolate, breakfast cereals, and pickled vegetables are stated as a source of acute nickel exposure especially for adults. Acute and chronic ingestion of these Ni sources results in eczematous flare-up reactions in the skin of Ni-sensitive individuals, in particular [10].

Moreover, the metal alloys of Ni with other elements occur in nature and frequently used in the industry, and the simultaneous increase in the levels of other elements with Ni has been reported by previous researchers. Also, the alloys of Ni with Pd, Cr, Cd, Al, Mn, Co, and Cu were used in the manufacturing of dental and orthopedic implants, kitchen utensils, inexpensive jewelry, coins, and other metal equipment. The increased levels of Cd, Pb, Sb, and Ba in smokers, Li, B, Mg, Al, P, K, Ca, Cr, Mn, Co, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, Pb, Bi, and U depending on the nutritional sources and occupational exposure have been observed together with the increased Ni concentrations in blood and urine samples [11–13].

Studies reported a prevalence of Ni sensitization of 10.9% in a population of healthy children, and dermal contact, ingestion, and inhalation have been reported as the main routes of exposure to the metal [14].

The main route of excretion for Ni is urine, whereas it can also be excreted in the breast milk during the lactation period. Experimental data derived from in vivo animal studies indicate that Ni does not accumulate in tissues following ingestion through the oral route [10]. The urinary excretion profile of the female patients without Ni dermatitis found that the 95th percentile of the cases examined had higher Ni levels than the upper limit of the reference interval determined by the German authorities, suggesting the age and nutritional sources as the essential contributors of the total metal accumulation [15].

Considering all these data, there is a need for a comprehensive study on the frequency of exposure to imitation jewelry and occupational equipment use that is proven to cause Ni sensitivity and the correlation of Ni-rich food intake and nutritional patterns with the Ni concentration and other toxic elements in the urine.

The main purpose of our study was to determine the concentration of Ni and other elements (Li, B, Mg, Al, P, K, Ca, Cr, Mn, Co, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, Pb, Bi, and U) in the urine samples of individuals whose Ni sensitivity has been proven dermatologically, in comparison with those without Ni sensitivity, and to determine the effect of imitation jewelry use, nicotine and alcohol consumption, and nutritional patterns with Ni-rich foods on these outcomes.

## Materials and Methods

### Chemicals and Equipment

The HNO<sub>3</sub> with a 65% purity was purchased from Merck (Merck KgaA, Darmstadt, Germany), and ICP-MS Tune Solution (10 µg/mL) and ICP-MS Calibration Standard (10 µg/mL) were manufactured by High-Purity Standards (Charleston, SC, USA). Certified urine reference material (CRM) was obtained from Seronorm (SERO, Billingstad, Norway). Indium (In) and Gallium (Ga) (1000 mg mL<sup>-1</sup>) standards as internal standards (ISs) were supplied from Absolute Standards Inc. (Hamden, CT, USA). All measurements were carried out using high purity argon gas (>99.999% purity, Okser, Turkey) to generate Ar plasma. Ultra-pure water (Millipore Direct-Q UV 3, Molsheim, France) (18.2 MΩ cm) was used for dilution of samples and standards.

A Thermo Scientific X Series II ICP-MS (Thermo Fisher Scientific, Bremen, Germany) coupled with a CETAC (Omaha, Neb., USA) auto-sampler unit (ASX 520) was used for elemental determinations. BPC BioSed Prime Automatic Photometer was used for the spectrophotometric analyses of the urine creatinine levels. The Inert Quadrant (IQ) Chamber test patches were provided from Chemotechnique MB Diagnostics AB (Vellinge, Sweden).

### Participants of the Study

The study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the institutional Clinical Research Ethics Committee (83045809/27009). Power analysis with 80% power and a significance level of  $p < 0.05$  determined a minimum sample size of 100 participants with and 200 subjects without Ni sensitivity.

All participants were informed about the study content and characteristics, and signed informed consent, and Participant Information Forms were obtained from all participants.

All voluntary participants were asked the information regarding their age, gender, occupation, smoking and alcohol consumption habits, and the types and frequency of imitation jewelry use, occupational contact with metal objects, sensitivity reactions such as itching and redness to the metal objects, and the frequency of consumption of Ni-rich foods. A questionnaire was provided, and participants were asked to mark one of the alternatives as follows for the evaluation of their frequency of ingestion of Ni-rich food sources such as cocoa, chocolate, apricot, hazelnut, almond, lentil, oat, and ready to use foods and drinks: Every day, once a week, once a month, occasionally, never. Qualitative results were obtained from participants instead the consumed amount of portion for related food or drink.

### Collection of Urine Samples

The urine samples of the participants were collected prior to the Ni patch test considering that there might be a trace amount of Ni absorption during the sensitivity test that might interfere with the study results.

Sterile, screw-cap, and pre-washed urine containers [with 2% HNO<sub>3</sub> (v/v)] were used for the collection of urine samples from the volunteers. The 1-mL aliquots of urine samples were kept at +4 °C until the day of the analyses.

Simultaneous analysis of 24 elements was performed using an ICP-MS-based analytical method with high accuracy and precision.

### Ni Patch Test

Following the physical examination at Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty, Department of Dermatology and Venerology, a Ni patch test was applied to the participants without a history of ongoing pregnancy, asthma, immune disease, and allergic contact dermatitis. The participants with topical or systemic use of corticosteroids within the last 2 weeks were also excluded.

The Inert Quadrante (IQ) Chamber test (Chemotechnique MB Diagnostics AB, Vellinge, Sweden) patches containing a certain concentration of Ni haptens were applied on a clean, dry, and hairless area of the dorsal region or forearm. The subjects were advised to keep the area dry for 48 h, to avoid excessive sweating and heavy lifting to prevent the patches from loosening, and test patches were removed after 48 h. At the end of the 48 h, the test area was marked with a pen and evaluated 30 min later. Positive reactions were recorded according to the Investigator Global Assessment Scale, and the participants were asked to keep the area dry until the second visit.

In order to detect an incidence of delayed-type allergic reactions, the test area was re-evaluated after 72 h. The patients with a positive reaction after 72 h were re-evaluated on

the 96th hour. The test results were assessed according to the formation of erythema, infiltration, papules, and /or vesicular reaction (Table 1) [16]. An example of a positive (++) patch test result was shown in Fig. 1.

### Preparation and Analysis of Urine Samples

The ICP-MS conditions and system configuration of the study are shown in Table 2.

The calibration solutions were prepared daily in 2% HNO<sub>3</sub> by the incremental amount as 0.1, 0.2, 0.5, 1, 2, 5, 10, 25, 50, 100, and 150 ng mL<sup>-1</sup> for Li, Al, Cr, Mn, Co, Ni, Cu, As, Se, Sr, Mo, Cd, Sb, Ba, Pb, Bi, and U; and 5, 10, 20, 50, 100, 200, 250, 300, and 400 ng mL<sup>-1</sup> for B, Mg, P, and Zn. Additionally, K, Ca, and Sn were analyzed with a semi-quantitative method since certified reference standards were not present for these elements. All calibration solutions and samples were added internal standards (20 ng mL<sup>-1</sup> of Ga and In) and prepared in triplicate for each experiment. The amounts of Ni and other elements in the samples were determined by the linear calibration curve obtained from the calibration solutions.

All polypropylene materials and vials with flat-top screw caps (Falcon, Fisher Scientific, UK) were cleaned by soaking them in 5% (v/v) HNO<sub>3</sub> solution, thoroughly rinsed with ultra-pure water and dried at room temperature before use.

Analytical experiments were performed using an ICP-MS-based method due to its acceptable range of repeatability, linearity, and accuracy with standardization. While the analytical performance of the study was assessed, the limit of detection (LoD) of the method was calculated as 3 times the standard deviation of the blank samples ( $n=10$ ) and calculated as 0.155 ng/mL. The limit of quantification (LoQ) was calculated as 1.706 ng/mL. The correlation coefficient of the calibration curve of nickel with a linear range of 0.1–150 ng/mL was obtained from repetitive analyses ( $r > 0.999$ ). The accuracy was tested using certified reference material with a certified concentration of 41.3 ng/mL (acceptable range: 24.7–57.9 ng/mL). The average of three repetitive analysis results for a total of 6 weeks was calculated as 40.39 ng/mL (SD = 4.30) with a 98% accuracy. The performance parameters of other analytes and the results of the validation analyses have been reported previously [17].

One mL of urine samples was homogenised and equilibrated to room temperature, and 20 ng/mL Indium and Gallium (Internal Standard) solutions were added and followed by vortex-mixing, diluted 10 times with 2% HNO<sub>3</sub> solution.

The creatinine levels of the urine samples were measured spectrophotometrically, and the results were obtained in mg/dL. The elemental concentrations obtained were calculated as the microgram per 1 gram of creatinine and

**Table 1** The international rating system of the patch test [16]

|     |                                                                                         |
|-----|-----------------------------------------------------------------------------------------|
| –   | Negative result with no reaction                                                        |
| +/– | Suspected reaction, minimal erythema                                                    |
| +   | Weak, non-vesicular reaction with erythema, infiltration, and possibly discrete papules |
| ++  | Strong positive vesicular reaction with erythema, infiltration, and papules             |
| +++ | Extreme positive reaction with erythema, infiltration, and confluent vesicles           |
| IR  | Irritant reaction including soap effect, vesicles, blister, necrosis                    |

relative analyte results were reported as  $\mu\text{g/g}$  creatinine, since random spot urine samples are used for the analyses and evaluate the elemental concentrations independently from kidney functions of each participant.

### Statistical Analysis

Statistical evaluation of the data was performed using the Windows-based SPSS 16.0 (SPSS for Windows, Version 16.0. Chicago, SPSS Inc.) program. The normality of the quantitative variables was evaluated with the Shapiro Wilk test. A Mann-Whitney *U* test was used to compare two independent groups, and a Spearman's correlation test was used to determine the relationship between the variables as non-parametric tests. The Kruskal-Wallis test was employed for the group comparisons of more than two independent variables that do not fit the normal distribution. Binary comparisons of the significant results in the Kruskal Wallis test were re-evaluated using the Mann Whitney *U* test with Bonferroni correction. A *p* value of  $<0.05$  was considered statistically significant.

**Fig. 1** An example of a positive (++) patch test result

### Results and Discussion

A total of 388 out of 405 participants were included in the study. Of the 388 participants, 239 were female (61.6%) and 149 (38.4%) were male. The average age of women was 28.6 (median = 24), and the average age of men was 30.6 (median = 24).

According to the Ni patch test results, 92 (23.7%) of the participants were found to have Ni sensitivity whereas 296 (76.3%) individuals had a negative Ni patch test result. The mean urine total Ni concentration of the 92 individuals with a positive Ni sensitivity test result was  $4.475 \mu\text{g/g}$  creatinine (median = 4.260; SD = 2.527), whereas the participants with a negative test result had a mean urine total Ni level of  $4.256 \mu\text{g/g}$  creatinine (median = 3.588; SD = 4.970 ( $p = 0.068$ )). When the subjects were subdivided into groups depending on the grade of the Ni patch test reactions, there was not a significant difference between the groups in terms of urine Ni concentration ( $p = 0.228$ ) (Table 3).

The mean urine Ni concentration of the female participants was  $4.852 \mu\text{g/g}$  creatinine (SD= 5.365), whereas male

**Table 2** ICP-MS conditions and configuration

|                               |                                 |
|-------------------------------|---------------------------------|
| Radio frequency power         | 1300W                           |
| Plasma gas flow               | 13 L/min                        |
| Auxiliary gas flow            | 0.8 L/min                       |
| Concentric nebulizer gas flow | 0.85 L/min                      |
| Sampling depth                | 95                              |
| Sweeps/replicate              | 100                             |
| Replicates/sample             | 3                               |
| Spray chamber temperature     | 3 °C                            |
| Sample and skimmer cones      | Nickel                          |
| Dwell time                    | 0.01 s                          |
| Sample uptake                 | 60 s                            |
| Washing                       | 45 s                            |
| Peristaltic pump speed        | 33 rpm                          |
| Autosampler                   | Cetac ASX-520 Autosampler       |
| Software                      | PlasmaLab, Version 2.5.11.321   |
| Plasma gas                    | Argon (%99.,999), Okser, Turkey |

**Table 3** Demographic data of the study individuals and Kruskal Wallis test results evaluating the relationship between participants' patch test results and urine nickel concentrations

|                          |                                |                                                     |               |           |          |
|--------------------------|--------------------------------|-----------------------------------------------------|---------------|-----------|----------|
| Age—female (years)       |                                | 28.6 (median = 24)                                  |               |           |          |
| Age—male (years)         |                                | 30.6 (median = 24)                                  |               |           |          |
| Female/Male              |                                | 239/149                                             |               |           |          |
| Nickel sensitivity       |                                |                                                     |               |           |          |
| Yes                      |                                | 92 (23.7%)                                          |               |           |          |
| No                       |                                | 296 (76.3%)                                         |               |           |          |
|                          |                                | <b>Urine nickel concentration (µg/g creatinine)</b> |               |           |          |
| Nickel patch test result |                                | <b>Mean</b>                                         | <b>Median</b> | <b>SD</b> | <b>p</b> |
|                          | <b>Negative</b> (n=296, 76.3%) | 4.256                                               | 3.588         | 4.971     | 0.228    |
|                          | <b>+</b> (n=21, 5.4%)          | 4.864                                               | 4.638         | 2.651     |          |
|                          | <b>++</b> (n=68, 17.5%)        | 4.405                                               | 4.055         | 2.549     |          |
|                          | <b>+++</b> (n=3, 0.8%)         | 3.335                                               | 3.325         | 0.029     |          |

subjects had a mean concentration of 3.435 µg/g creatinine (SD= 2.383) urinary Ni. The urine Ni concentration was significantly different between female and male participants ( $p=0.001$ ,  $z=4.889$ ). The higher urine Ni concentration in females might be a consequence of their daily habitual exposure to cosmetic products, metal objects, water, and foods. It was reported that allergic contact dermatitis due to the exposure to Ni-containing objects is more common in female subjects [18].

The detailed information regarding the participants' status of smoking and alcohol consumption, occupational contact with metal objects, and the comparison of urinary Ni concentrations between the groups are presented in Table 4. We observed a significant difference in urine Ni concentration between the groups with and without prolonged occupational contact with metal objects, and regular alcohol intake. The mean urine Ni

concentration (3.717 µg/g creatinine) of individuals without metal object contact was significantly lower than those with object contact (4.619 µg/g creatinine) ( $p=0.003$ ).

The mean urine Ni concentration of those who used imitation jewelry regularly was significantly higher than those who did not ( $p=0.007$ ) (Table 5). The concentration of Ni in the urine of 159 participants, who stated that the use of imitation jewelry caused sensitivity symptoms was significantly higher compared to the group without a reported incidence of sensitivity to imitation jewelry ( $p=0.000$ ).

While the individuals were categorized into subgroups in terms of frequency of penetrating imitation jewelry use, the urine Ni concentrations were found to be different between the subjects for earring and nose ring ( $p=0.043$ ;  $p=0.017$ , respectively). However, a Bonferroni correction yielded a lack of significance between the paired study groups.

**Table 4** Evaluation of urine nickel concentrations according to the life habits of participants

|                                         | Urine nickel concentration (µg/g creatinine) |        |       | <i>p</i> (95% CI)                |
|-----------------------------------------|----------------------------------------------|--------|-------|----------------------------------|
|                                         | Mean                                         | Median | SD    |                                  |
| Female ( <i>n</i> =239, 61.6%)          | 4.852                                        | 4.124  | 5.365 | <b>0.001</b> ( <i>z</i> = 4.889) |
| Male ( <i>n</i> =149, 38.4%)            | 3.435                                        | 2.953  | 2.384 |                                  |
| Occupational contact with metal objects |                                              |        |       |                                  |
| No ( <i>n</i> =134, 34.5%)              | 3.717                                        | 3.183  | 2.640 | <b>0.003</b> ( <i>z</i> = 2.941) |
| Yes ( <i>n</i> =254, 65.5%)             | 4.62                                         | 4.016  | 5.214 |                                  |
| Smoking habit                           |                                              |        |       |                                  |
| Yes ( <i>n</i> =90, 23.2%)              | 4.254                                        | 3.579  | 3.018 | 0.928                            |
| No ( <i>n</i> =298, 76.8%)              | 4.324                                        | 3.825  | 4.877 |                                  |
| Alcohol intake                          |                                              |        |       |                                  |
| Yes ( <i>n</i> =75, 19.3%)              | 3.515                                        | 3.130  | 2.019 | <b>0.034</b> ( <i>z</i> = 2.116) |
| No ( <i>n</i> =313, 80.7%)              | 4.498                                        | 3.846  | 4.907 |                                  |

*p* values in bold form indicate the statistically significant results

**Table 5** Evaluation of urine nickel concentrations according to the imitation jewelry use patterns of participants

|                                                  | Urine nickel concentration (µg/g creatinine) |        |       | <i>p</i> (95% CI)                    |
|--------------------------------------------------|----------------------------------------------|--------|-------|--------------------------------------|
|                                                  | Mean                                         | Median | SD    |                                      |
| Frequent use of imitation jewelry                |                                              |        |       |                                      |
| Yes ( <i>n</i> =175, 45.2%)                      | 4.978                                        | 4.09   | 6.252 | <b>0.007</b> ( <i>z</i> = 2.710)     |
| No ( <i>n</i> =213, 54.8%)                       | 3.763                                        | 3.386  | 2.136 |                                      |
| Sensitivity reactions with imitation jewelry use |                                              |        |       |                                      |
| Yes ( <i>n</i> =159, 41.0%)                      | 5.045                                        | 4.227  | 6.33  | <b>&lt;0.000</b> ( <i>z</i> = 3.535) |
| No ( <i>n</i> =159, 41.0%)                       | 3.796                                        | 3.278  | 2.476 |                                      |
| Type of the frequently used imitation jewelry    |                                              |        |       |                                      |
| Earring                                          |                                              |        |       |                                      |
| Yes ( <i>n</i> =128, 33.0% )                     | 4.922                                        | 4.05   | 6.853 | <b>0.043</b> ( <i>z</i> = 2.019)     |
| No ( <i>n</i> =260, 67.0%)                       | 4.00                                         | 3.559  | 2.663 |                                      |
| Nose ring                                        |                                              |        |       |                                      |
| Yes ( <i>n</i> =4, 1.03%)                        | 7.651                                        | 7.429  | 3.122 | <b>0.017</b> ( <i>z</i> = 2.389)     |
| No ( <i>n</i> =384, 98.97%)                      | 4.273                                        | 3.640  | 4.513 |                                      |
| Ring                                             |                                              |        |       |                                      |
| Yes ( <i>n</i> =98, 25.3%)                       | 5.086                                        | 4.091  | 7.822 | 0.191                                |
| No ( <i>n</i> =290, 74.7%)                       | 4.045                                        | 3.588  | 2.530 |                                      |
| Watch                                            |                                              |        |       |                                      |
| Yes ( <i>n</i> =86, 22.2%)                       | 4.593                                        | 3.919  | 3.162 | 0.213                                |
| No ( <i>n</i> =302, 77.8%)                       | 4.227                                        | 3.618  | 4.828 |                                      |
| Piercing                                         |                                              |        |       |                                      |
| Yes ( <i>n</i> =2, 0.5%)                         | 4.201                                        | 4.201  | 1.945 | 0.738                                |
| No ( <i>n</i> =386, 99.5%)                       | 4.309                                        | 3.674  | 4.522 |                                      |
| Bracelet                                         |                                              |        |       |                                      |
| Yes ( <i>n</i> =21, 5.4%)                        | 4.016                                        | 3.312  | 2.761 | 0.638                                |
| No ( <i>n</i> =367, 94.6%)                       | 4.325                                        | 3.721  | 4.593 |                                      |

*p* values in bold form indicate the statistically significant results

In addition, the Ni concentrations in the urine samples of the participants who used penetrating jewelry such as earrings and piercings were found to be significantly higher ( $p=0.043$ ,  $p=0.017$ , respectively). This suggests that the metal objects that are in close contact with body fluids such as sweat and serum via the penetration surface, yield a higher level of corrosion, resulting in the migration of Ni into the organism. Therefore, control studies on Ni release should be employed more thoroughly for these types of jewelry. Although the EU Ni regulations Directive 94/27/EC mandate the analysis of imitation jewelry objects using a standardized synthetic sweat assay and restricted the amount of Ni released from jewelry and other products in contact with the skin to a maximum of 0.5 g/cm<sup>2</sup>/week, and 0.2 g/cm<sup>2</sup>/week for penetrating objects and jewelry such as earring and piercings, recent studies revealed the presence of Ni-containing jewelry above the EU limits in the markets [19, 20].

Furthermore, the EU directive is not in charge in most Asian countries, which are the main manufacturing source of

these objects. A recent study from Thailand revealed that 39.2% of the items in the Thai markets including belt buckles, bracelets, earrings, necklaces, rings, and watches gave a positive dimethylglyoxime test indicating a higher concentration of Ni in these products, and belt buckles and earrings were the main sources of exposure to Ni [21].

A recent artificial sweat matrix study, employing the imitation earrings from Turkish markets reported that 31.80% of earrings had a Ni content over the limit of European Commission Directive's migration limit with a minimum and maximum Ni release amounts of 0.21 and 30.11  $\mu\text{g}/\text{cm}^2/\text{week}$  [17].

Besides, Dimethylglyoxime test results of various types of cheap jewelry yielded a %24.9 incidence of Ni positivity, particularly in the objects with a lower price tag. The Ni detection ratio was significantly lower in jewelry obtained from branded chain stores [22].

However, Garg et al. showed that the prevalence of Ni allergy and hand eczema decreased significantly after Ni regulation in young Danish women and young Danish female

dermatitis patients [7]. An Italian study reported a high prevalence of Ni dermatitis and the number of earring with a higher Ni content exceeding the allowable migration limit, with a decreasing time trend following the implementation of the EU Ni Directive [23].

Oral intake of Ni via food and drinking water has been shown to be related to Ni-induced dermatitis reactions in sensitive individuals. Whole wheat, grain, rye, oat, cocoa, chocolate, tea, nuts, and legumes are the food sources with higher amounts of Ni [24, 25].

While we compared the mean urine Ni concentrations of those who consumed Ni-rich foods “apricot-hazelnut-almond” daily with the subjects who consume this group of nutrients once a week using the Kruskal-Wallis test, the frequency of this type of food source was significantly related to the urine Ni concentrations ( $p = 0.022$ ) (Table 6). We did not find a correlation between the ingestion frequency of cocoa, chocolate, lentil, oat and ready-to-use foods and drinks, and urine Ni concentrations ( $p = 0.361$ ;  $p = 0.259$ ;  $p = 0.125$ ;  $p = 0.228$ , respectively).

Environmental studies reported the Ni concentrations in the soil as 5–500  $\mu\text{g/g}$  and in plant tissue as 0.5–5  $\mu\text{g/g}$  which may be higher locally in industrialized and urbanized regions, whereas 0.1–5  $\mu\text{g/g}$  in animal tissue, and 5–100  $\mu\text{g/L}$  in freshwater [26, 27].

Since Ni was shown to be essential for the catalytic activity of some plant enzymes, and all these sources are in reciprocal interaction, the content of the Ni in the consumed food would be affected by the Ni concentration where the food sources were originated [28].

The high accuracy, recovery, and repeatability data obtained from the analysis results indicate that this study is efficient and appropriate for the quantitative determination of Ni and other elements in urine samples.

Since the ICP-MS system is a reliable and convenient method for the determination of elemental Ni and Li, B, Mg, Al, P, K, Ca, Cr, Mn, Co, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, Pb, Bi,

and U with high precision at very low concentrations, the implementation of this system in our study has ensured that the intended targets were achieved without interference.

The analyses were performed without any interference from other relevant elements, and all experiments were performed within the linear range of the method.

Studies from different countries on Ni release reported the relationship between Ni-containing objects and nutrients and allergic contact dermatitis. Kasper-Sonnenberg et al. investigated the relationship between Ni allergy and Ni concentration in urine, ambient air, and tap waters used by the study subjects, 749 children at the age of 6 years, and their mother residing close to a steel factory [14].

They reported that the concentration of urinary Ni in both mother and child was high while an increased Ni concentration was present in the ambient air. In addition, they concluded that there was a relationship between the Ni sensitivity rate of these individuals and the concentration of Ni in the ambient air close to the steel factory.

In their atomic absorption spectrometry-based study, Darsow et al. reported that 95% of the 164 female participants without a history of contact dermatitis had a urinary Ni concentration above the reference value of 3  $\mu\text{g/L}$ , and they concluded that the nutrition had an effect on urine Ni concentrations, which presented an increasing trend with the age [15].

We did not observe a correlation between the age and urine Ni concentration ( $r = 0.005$ ,  $p = 0.919$ ). The reason for the lack of correlation between the urine Ni levels and age might be due to the relatively younger age of participants in our study group. Since hypersensitivity reactions occur with an increasing amount of encountered antigens during the life span, an older sampling group might provide different correlation results.

Ni is frequently used in the production of imitation jewelry and metal objects due to its inexpensive, light, and corrosion-resistant properties, and released from these materials alongside the copper, iron, cobalt, and zinc. In addition, simultaneous allergic reactions to Ni and other elements have been

**Table 6** The data of study participants depending on the types and the relationship between consumption frequency of foods with high nickel content and urine nickel concentration ( $n = 388$ )

| Frequency of consumption    | Type of nutrient with Nickel-rich content |                                   |                |             |                                                       |
|-----------------------------|-------------------------------------------|-----------------------------------|----------------|-------------|-------------------------------------------------------|
|                             | Cocoa, chocolate ( $n$ )                  | Apricot, hazelnut, almond ( $n$ ) | Lentil ( $n$ ) | Oat ( $n$ ) | Ready to use (foods, tea, canned foods, etc.) ( $n$ ) |
| Every day                   | 166                                       | 76                                | 22             | 27          | 199                                                   |
| Once a week                 | 132                                       | 152                               | 187            | 62          | 85                                                    |
| Once a month                | 40                                        | 99                                | 113            | 81          | 28                                                    |
| Occasionally                | 38                                        | 51                                | 53             | 110         | 54                                                    |
| Never                       | 12                                        | 10                                | 13             | 108         | 22                                                    |
| Kruskal Wallis test ( $p$ ) | 0.361                                     | <b>0.022</b>                      | 0.259          | 0.125       | 0.228                                                 |

$p$  values in bold form indicate the statistically significant results

**Table 7** Spearman's correlation test results of the elements in the urine samples of the study group ( $n=388$ )

|           | Li     | B      | Mg     | Al      | P       | K       | Ca      | Cr      | Mn      | Co    | Ni      | Cu     | Zn      | As     | Se      | Sr     | Mo     | Cd      | Sn      | Sb      | Ba     | Pb     | Bi   | U    |
|-----------|--------|--------|--------|---------|---------|---------|---------|---------|---------|-------|---------|--------|---------|--------|---------|--------|--------|---------|---------|---------|--------|--------|------|------|
| <b>Li</b> | 1.00   |        |        |         |         |         |         |         |         |       |         |        |         |        |         |        |        |         |         |         |        |        |      |      |
| <b>B</b>  | .550** | 1.00   |        |         |         |         |         |         |         |       |         |        |         |        |         |        |        |         |         |         |        |        |      |      |
| <b>Mg</b> | .404** | .331** | 1.00   |         |         |         |         |         |         |       |         |        |         |        |         |        |        |         |         |         |        |        |      |      |
| <b>Al</b> | .245** | .234** | .322** | 1.00    |         |         |         |         |         |       |         |        |         |        |         |        |        |         |         |         |        |        |      |      |
| <b>P</b>  | .239** | .268** | .376** | .104*   | 1.00    |         |         |         |         |       |         |        |         |        |         |        |        |         |         |         |        |        |      |      |
| <b>K</b>  | .309** | .302** | .416** | .144**  | .427**  | 1.00    |         |         |         |       |         |        |         |        |         |        |        |         |         |         |        |        |      |      |
| <b>Ca</b> | .261** | .145** | .608** | .160**  | .279**  | .367**  | 1.00    |         |         |       |         |        |         |        |         |        |        |         |         |         |        |        |      |      |
| <b>Cr</b> | .334** | .509** | .216** | .295**  | .209**  | .113*   | .106*   | 1.00    |         |       |         |        |         |        |         |        |        |         |         |         |        |        |      |      |
| <b>Mn</b> | .239** | .269** | .181** | .322**  | .115*   | .130*   | .060    | .375**  | 1.00    |       |         |        |         |        |         |        |        |         |         |         |        |        |      |      |
| <b>Co</b> | .040   | -.012  | .012   | -.040   |         | -.039   | .056    | .023    | -.022   | 1.00  |         |        |         |        |         |        |        |         |         |         |        |        |      |      |
| <b>Ni</b> | .281** | .341** | .344** | .304**  | .118*   | .119*   | .250**  | .385**  | .019    | .120* | 1.00    |        |         |        |         |        |        |         |         |         |        |        |      |      |
| <b>Cu</b> | .439** | .469** | .333** | .464**  | .276**  | .207**  | .088    | .659**  | .451**  | -.001 | .374**  | 1.00   |         |        |         |        |        |         |         |         |        |        |      |      |
| <b>Zn</b> | .070*  | .011** | .055** | -.150** | .056    | .068    | .087    | -.150** | -.015   | -.022 | -.039** | .030** | 1.00    |        |         |        |        |         |         |         |        |        |      |      |
| <b>As</b> | .410** | .428** | .217** | .159**  | .209**  | .244**  | .101*   | .305**  | .126*   | -.056 | .290**  | .430** | .076*   | 1.00   |         |        |        |         |         |         |        |        |      |      |
| <b>Se</b> | .391** | .542** | .299** | .257**  | .304**  | .170**  | .097    | .686**  | .310**  | .000  | .324**  | .722** | .014**  | .519** | 1.00    |        |        |         |         |         |        |        |      |      |
| <b>Sr</b> | .391** | .319** | .588** | .224**  | .136**  | .139**  | .580**  | .436**  | .204**  | .104* | .498**  | .352** | .002**  | .234** | .376**  | 1.00   |        |         |         |         |        |        |      |      |
| <b>Mo</b> | .262** | .285** | .163** | .077    | .083    | .076    | .071    | .319**  | .087    | .056  | .308**  | .312** | -.011** | .187** | .371**  | .289** | 1.00   |         |         |         |        |        |      |      |
| <b>Cd</b> | .195** | .295** | .155** | .336**  | .180**  | .109*   | .044    | .298**  | .039    | .059  | .293**  | .446** | -.039** | .218** | .348**  | .148** | .395** | 1.00    |         |         |        |        |      |      |
| <b>Sn</b> | .118*  | .152** | .021   | -.104*  | .113*   | .057    | .039    | -.041   | -.204** | .079  | .339**  | -.017  | .111*   | .166** | .093    | .147** | .198** | .097    | 1.00    |         |        |        |      |      |
| <b>Sb</b> | .080   | -.075  | .110*  | -.161** | .064    | .172**  | .163**  | -.274** | .086    | .017  | -.061   | -.093  | .671**  | -.036  | -.151** | .015   | -.060  | -.161** | .176**  | 1.00    |        |        |      |      |
| <b>Ba</b> | .142** | .061   | .270** | .238**  | .043    | .037    | .271**  | .179**  | .179**  | .000  | .280**  | .234** | .047    | .068   | .158**  | .389** | .171** | .085    | .132**  | .004    | 1.00   |        |      |      |
| <b>Pb</b> | .159** | .216** | .104*  | .342**  | .122*   | .052    | .023    | .368**  | .112*   | .079  | .280**  | .365** | -.147** | .137** | .341**  | .175** | .217** | .323**  | .254**  | -.100*  | .135** | 1.00   |      |      |
| <b>Bi</b> | .199** | .185** | -.018  | .076    | -.141** | -.142** | -.197** | .185**  | .307**  | .124* | .128*   | .316** | .241**  | .236** | .290**  | .137** | .106*  | .103*   | -.092   | .178**  | .039   | .084   | 1.00 |      |
| <b>U</b>  | .073   | .166** | -.114* | .190**  | -.065   | -.306** | -.228** | .487**  | .246**  | .114* | .080    | .361** | -.283** | .017   | .301**  | .109** | .124*  | .127*   | -.143** | -.333** | .053   | .249** | .067 | 1.00 |

\*\*Correlation is significant at the 0.01 level (two-tailed)

\*Correlation is significant at the 0.05 level (two-tailed)

reported [28–30]. Thus, we correlated the levels of these elements alongside other metals with the urine Ni concentrations in our study group. We found a significant correlation between Ni-Li, Ni-B, Ni-Mg, Ni-Al, Ni-P, Ni-K, Ni-Ca, Ni-Cr, Ni-Mn, Ni-Cu, Ni-Zn, Ni-As, Ni-Se, Ni-Sr, Ni-Mo, Ni-Cd, Ni-Ba, Ni-Pb, Ni-Bi, Ni-Co alongside other various element pairs (Table 7). The positive correlation with other metals in urine samples suggests a close interaction between Ni and other metals both in the structure of occupational equipment, jewelry, and metal accessories, as well as the potential environmental sources. Studies showed increased levels of Cd, Pb, Ba, and Sb in smokers, and excretion routes of these elements differ in the organism [11, 31]. In addition, the half-lives and the exposure durations of metals would yield diversified outcomes in different study set-ups. It has also been suggested that, blood Cd indicates recent exposure and urinary Cd is higher in chronic intake of or contact to the element [32]. Ni is a naturally occurring element in nature, and the main potential sources of other elements in correlation with Ni are food and drinking water for non-smokers. Hence, a diet rich in vegetables and soil-grown foods would be the main origin of the correlation of these metals in the urine samples. Noteworthy is that, the concentrations of urine Mo, Zn, Cd, and Cu are significantly affected by fasting; thus, the sampling conditions for the metal analyses should be uniform and well-designed [33].

Our study has several limitations to declare. We evaluated occupational exposure through the personal reports of the subjects and did not perform an analysis evaluating the level of their exposure during the work time. We also did not analyze the Ni content in the ambient air of the participants' residential areas; however, all participants were residing in one of Europe's largest metropolis. Since dental braces and alloys, orthopedic joint replacement, and heart and endovascular prostheses are reported to be associated with increased Ni sensitivity, we did not question the exposure of our study subjects to those materials. We collected single urine samples from the participants, and various half-life durations have been reported following the exposure to different types of Ni-containing products [34]. We did not evaluate the consumption of Ni-rich nutrients in terms of tolerable daily intake (TDI) and tolerable upper intake level (UL); hence, proper body weight-related calculations might yield different results. Beside, blood samples of participants or consumed foods and drinks could also be analyzed to determine the nickel or other elements to see the big picture for inorganic contamination. Hence, further studies investigating the concerned limitations of our study might yield beneficial results for a better understanding of the effect of elemental exposure on allergic contact dermatitis and other atopic reactions.

In conclusion, to our knowledge, this study is the first population-based cross-sectional study to examine the relationship between Ni sensitivity and Ni concentration

alongside other excreted elements in urine with a validated ICP-MS-based method. The employment of a wide range of elemental profile analyses in the urine samples would provide a better understanding of the excretion pattern of these elements depending on smoking, jewelry use, nutritional habits, and continuous contact with metals. Guidelines and regulations suggest a thorough control and validation of the Ni content of imitation objects and materials that are contacted through the occupation owing to its dermatological and long-term carcinogenic effects. Our study reported that urine Ni concentrations are closely related to the frequency of exposure to Ni-containing objects and jewelry, particularly via penetrating objects, and the presence of a patch test-proven sensitivity reaction to Ni. Public awareness, regular supervision, and employment of regulations should be addressed in an attempt to achieve a better life quality for the people with hypersensitivity reactions and prevent possible adverse effects in the population.

**Funding** Authors would like to thank the Scientific and Technological Research Council of Turkey (TUBITAK) and Scientific Research Fund of Istanbul University-Cerrahpaşa, Turkey, for support of this work with the Project numbers 213S098 and BYP-54488, respectively.

## Declarations

**Conflict of Interest** The authors declare no competing interests.

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