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# Investigation of the potential use of VCAM-1, TNF-α, IL-10 and IL-6 as biomarkers of nickel exposure

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## ABSTRACT

**Objectives**: Industrial and agricultural activities such as mining, smelting, and the discharging of industrial and domestic wastewater have increased the severity of heavy metal pollution in environments. Nickel poisoning continues to be an important occupational health problem in many branches of industry, especially coating. Occupational exposure to nickel can occur through skin contact or inhalation of nickel-containing aerosols, dust, or fumes. As a result of the toxic effect of nickel, it can cause various health problems, including respiratory and dermatological effects.

**Material and Method**: The study included 56 male workers exposed to nickel in a coating factory (Ni-exposed group) and 44 non-exposed male workers (control group). Vascular Cell Adhesion protein (VCAM)-1, Tumor Necrosis Factor (TNF)-α, Interleukin (IL)-10, and IL-6 levels of serum were analyzed using enzyme-linked immunosorbent assays (ELISA). Ni levels were determined using inductively coupled plasma mass spectrometry (ICP-MS) in urine samples.

**Results**: Significant intergroup differences were observed in the levels of all inflammatory parameters such as VCAM-1, TNF-α, IL-10 and IL-6 ($p<0.01$ for all).

**Conclusions**: The correlations between increased inflammatory biomarkers levels and exposed/control groups suggest a close relationship between inflammation and toxicity. This relationship provides a clinical model for the early diagnosis of nickel toxicity.

**Keywords**: Nickel exposure, coating workers, inflammation parameters, early diagnosis

## INTRODUCTION

Heavy metals such as nickel are highly conservative and refractory elemental pollutants that produce irreversible physiological and biochemical changes in organisms. Industrial and agricultural activities such as mining, smelting, and the discharging of industrial and domestic wastewater have increased the severity of heavy metal pollution in environments (1). The International Agency for Research on Cancer classified nickel compounds as group 1, carcinogenic to humans (2). Occupational exposure to nickel can occur through skin contact or inhalation of nickel-containing aerosols, clouds of dust, or fumes. (3). Nickel poisoning continues to be an important occupational health problem in many branches of industry (4). Nickel toxicity may result with various health problems, including respiratory and dermatological effects (2). Nickel is not metabolized and poorly absorbed through the skin, which is eliminated via the urine (3). Nickel at a low level in biological and environmental specimens has become a critical research topic in terms of community health (5).

The advances achieved with biological-analytical techniques in the field of biomarkers accelerate the studies on the effectiveness of exposure to chemical agents, individual or population susceptibility, risk assessments, the dose-response relationship for chemicals and treatments (6). Tumor Necrosis Factor (TNF-α) is one of the pro-inflammatory cytokines mediating immune regulation (7,8). TNF-alpha has also been suggested to be an endogenous tumor promoter (9). Like TNF- α, Interleukin (IL)-6 plays an important pathophysiological role not only in inflammatory diseases but also in cancers. However, Vascular Cell Adhesion protein (VCAM-1), TNF-α, IL-10 and IL-6 especially play an important role in the interpretation of systemic inflammatory responses (7–11).

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The correlations between increased inflammatory biomarkers levels and exposed/control groups suggest a close relationship between inflammation and toxicity. This relationship provides a clinical model for the early diagnosis for nickel toxicity. We aimed to evaluate the toxicity of nickel in Turkish coating workers. The research investigated the potential use of VCAM-1, TNF-α, IL-10 and IL-6 as biomarkers of nickel exposure and provides a clinical model for the inflammation that can be caused by nickel toxicity among the exposed workers in the coating industry.

## MATERIAL AND METHOD

This study was conducted with the approval of Bozok University Ethics Committee (Date: 2.10.2016; Decision No: 69). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study included 56 male workers exposed to nickel in coating factories (Ni-exposed group) and 44 non-exposed male workers (control group). Nickel-exposed workers were selected among workers who are under risk of various toxic metals, including arsenic and cadmium. The exclusion criteria for all groups were acute infections (physical examination and/or imaging), chronic lung disease, diabetes mellitus, diagnosed coronary vascular disease, hypertension, rheumatic diseases, or cancer.

### Collection of Serum Samples and VCAM-1, TNF-α, IL-10 and IL-6 Analysis

The serum was separated from blood samples by centrifugation at 1500 rpm for 10 min and then transferred to 2 mL Eppendorf tubes and frozen to -20 °C until analysis. For VCAM-1, TNF-α, IL-10 and IL-6 analyses were used in the respective ELISA kits and prepared the samples following manufacturer's instructions for each kit. The samples were then placed on microplates and analyzed using enzyme-linked immunosorbent assays (ELISA-BMG LABTECH, CLARIOstar model). The wavelength was set at 450 nm and r2 values, TNF-α, IL-10 and IL-6 of the calibration curves were obtained for VCAM-1 0.9993, for TNF-α 0.9998, for IL-10 0.9993 and for IL-6 0.9995 (11).

### Collection of Urine Samples and Ni Analysis

For nickel analysis, 1 mL amounts of the urine samples were added to the Teflon tubes and then 5 ml 65% nitric acid and 5 ml ultra-pure water added to the tubes. For digestion sample was used Milestone microwave digestion system. After digestion, samples were transferred to 50 mL polypropylene tubes, added ultra-pure water to obtain the total volume of 20 mL, and stored at +4 °C until analysis (11). Ni levels were determined using inductively coupled plasma mass spectrometry (ICP-MS). The operating parameters of ICP-MS were set as follows: RF power 1550 W, nebulizer gas 0.90 L/min, plasma gas 0.80 L/min, nebulizer pressure 2.9 bar, dwell time 0.01 and spray chamber temperature 3.0°C. The sampler probe was washed between injections by rinsing with ultrapure water for 30 s, followed by washing with 2% HNO3 for 45 s then rinsing with ultrapure water for 45 s. After the wash steps, the instrument automatically ran the next sample. The r2 value of the calibration curve was calculated as 0.9998 and the interval of the calibration was set at 0.1–1000 µg/L nickel. Sample and standard of measurements were repeated three times. Method validations were performed with CRM-Seronorm™

Trace Elements Whole Blood L-2. CRM was measured 5 times on the same day and on different days. Moreover, the average of the repeated measurements was used for the validation of the method whereby the relative standard deviation (RSD) of the values did not exceed 5%. Coefficient of variation (CV) and recovery was found as %3.58 and %103.32, respectively. On the other hand, ICP-MS method for Ni analysis provided limit of detection (LOD) and lowest limit of quantification (LOQ) equal to 0.022 and 0.137, respectively.

### Statistical Analysis

The SPSS 20.0 software was used in statistical analysis. The suitability of the parameters to the normal distribution was evaluated with the Kolmogorov Smirnov test. It was observed that the data were normally distributed and parametric tests were applied. Continuous variables were presented with their mean and standard deviations. The difference between the two means was evaluated with the t-test, and the relations of the variables with each other were evaluated with Pearson Correlation analysis.

## RESULTS

This study included 100 male subjects, who were stratified into a control group of 44 subjects and a Ni-exposed group of 56 subjects. The mean age and BMI values of the control group and the exposure group were similar ($p>0.05$). Significant intergroup differences were observed in the levels of all inflammatory parameters such as VCAM-1, TNF-α, IL-10 and IL-6 ($p<0.01$ for all). Nickel levels in VCAM-1, TNF-α, IL-10 and IL-6 parameters were significantly higher in the exposure group. The relationships between the main parameters in all groups are presented in **Table 1**.

The positive correlations were found between Ni and VCAM-1 ($r=0.704$), TNF-α ($r=0.697$), IL-10 ($r=0.640$), and IL-6 ($r=0.268$) ($p<0.01$ for all). Among them, the strongest association was found between Ni and VCAM-1 ($p<0.01$). The IL-6 level also negatively correlated with the hemoglobin (HGB) ($r= -0,257$, $p<0.01$) and

**Table 2.** Pearson correlation coefficients of all tested parameters.

| | BMI | WBC | HGB | HCT | PLT | ALT | AST | Ni Levels | VCAM-1 | TNF-a | IL-10 | IL-6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | 0,333** | -0,065 | 0,013 | -0,04 | 0,045 | -0,11 | -0,085 | 0,042 | -0,091 | -0,085 | -0,092 | -0,330** |
| BMI | 1 | -0,133 | -0,003 | -0,01 | -0,101 | 0,177 | 0,104 | 0,007 | 0,084 | 0,17 | 0,182 | -0,072 |
| WBC | | 1 | 0,107 | 0,1 | 0,279** | 0,036 | 0,004 | 0,024 | 0,004 | -0,044 | -0,08 | 0,063 |
| HGB | | | 1 | 0,913** | -0,028 | 0,235* | 0,156 | 0,095 | 0,009 | -0,011 | -0,028 | -0,257** |
| HCT | | | | 1 | -0,16 | 0,239* | 0,131 | 0,058 | -0,029 | -0,092 | -0,09 | -0,238* |
| PLT | | | | | 1 | 0,18 | 0,147 | -0,065 | 0,088 | -0,065 | -0,134 | 0,04 |
| ALT | | | | | | 1 | 0,707** | 0,137 | 0,113 | 0,097 | 0,027 | -0,009 |
| AST | | | | | | | 1 | 0,138 | 0,032 | 0,046 | 0,035 | -0,082 |
| Ni Levels | | | | | | | | 1 | 0,704** | 0,697** | 0,640** | 0,268** |
| VCAM-1 | | | | | | | | | 1 | 0,798** | 0,577** | 0,420** |
| TNF-a | | | | | | | | | | 1 | 0,888** | 0,418** |
| IL-10 | | | | | | | | | | | 1 | 0,408** |

**Table 1.** Differences in parameters between control and Ni-exposed groups (n=100).

| | Groups | N | Mean | Std. Deviation | t | p |
|---|---|---|---|---|---|---|
| Age (years) | Control | 44 | 36.57 | 10.05 | 0.724 | 0.471 |
| | Ni-Exposed | 56 | 38.00 | 9.64 | | |
| BMI ($kg/m^2$) | Control | 44 | 26.85 | 2.59 | 1.303 | 0.196 |
| | Ni-Exposed | 56 | 27.61 | 3.09 | | |
| WBC (µl/ml) | Control | 44 | 7.62 | 1.92 | 0.416 | 0.678 |
| | Ni-Exposed | 56 | 7.78 | 1.81 | | |
| HGB (g/dL) | Control | 44 | 15.11 | 1.48 | 0.663 | 0.509 |
| | Ni-Exposed | 56 | 15.32 | 1.64 | | |
| HCT (%) | Control | 44 | 45.31 | 3.83 | 0.335 | 0.738 |
| | Ni-Exposed | 56 | 45.57 | 3.76 | | |
| PLT ($10^3$/µL) | Control | 44 | 243.25 | 49.66 | 1.182 | 0.240 |
| | Ni-Exposed | 56 | 230 | 59.92 | | |
| ALT (IU/L) | Control | 44 | 22.48 | 12.23 | 1.656 | 0.101 |
| | Ni-Exposed | 56 | 27.48 | 16.85 | | |
| AST (IU/L) | Control | 44 | 19.84 | 4.93 | 0.872 | 0.385 |
| | Ni-Exposed | 56 | 20.86 | 6.37 | | |
| Ni (µg/L) | Control | 44 | 1.35 | 0.83 | 19.145 | <0.001* |
| | Ni-Exposed | 56 | 5.86 | 1.49 | | |
| VCAM-1 (ng/mL) | Control | 44 | 4.41 | 1.91 | 10.545 | <0.001* |
| | Ni-Exposed | 56 | 12.21 | 5.1 | | |
| TNF-a (pg/mL) | Control | 44 | 2.96 | 1.36 | 9.832 | <0.001* |
| | Ni-Exposed | 56 | 5.21 | 0.76 | | |
| IL-10 (pg/mL) | Control | 44 | 30.67 | 8.69 | 7.972 | <0.001* |
| | Ni-Exposed | 56 | 41.8 | 3.62 | | |
| IL-6 (pg/mL) | Control | 44 | 25.57 | 12.4 | 3.114 | 0.002* |
| | Ni-Exposed | 56 | 34.49 | 16.24 | | |

hematocrit (HCT) levels (r= -0,238, p<0.01) (**Table 2**).

## DISCUSSION

As a very common metal used in alloys, nickel can be found in many forms such as metallic nickel, nickel alloy, oxidic nickel, soluble nickel, sulfidic nickel and nickel carbonyl. The major route of occupational nickel intake is oral, inhalation and dermal absorption. The contamination of nickel among workers depends upon several factors: aerodynamic size of particles, the concentration of nickel that is inhaled, ventilation rate of worker, the proper use of personal protective equipment, and the safety awareness of the worker. While the acute effects of nickel exposure are mostly associated with nickel carbonyl, chronic effects can be seen in all nickel forms, especially metallic nickel, and the most prominent chronic effects are lung and sinonasal cancers (12,13).

Nickel-induced carcinogenesis was found to be related to epigenetic changes, inflammation and generation of reactive oxygen species in many studies (14,15). In a study with nickel oxide nanoparticles, it was demonstrated that inflammation is triggered in lung epithelial cells and genotoxic effects may occur accordingly (16). In another study, 0.2 mg of nickel oxide were given intratracheally and rats were sacrificed from three days to six months. A persistent increase of macrophage inflammatory protein (MIP-1α) and a transient expression increase of interleukin-1α (IL-1α), interleukin-1β (IL-1β) and macrophage inflammatory protein-1 (MIP-1) were observed (17). As a representative of the β- or C-C chemokines, MIP-1 is induced by inflammation, IL-1 and TNF-α. MIP-1 has an important modulating role in the development of inflammatory response especially during infection by regulating cytokine production and recruiting mononuclear cells (18). In an experimental study with male Sprague-Dawley rats, Ni Cl2 were found to make immunologic effects, but suppressed IL-10 dose- and time-dependently (19).

In our study, a positive correlation was found between Ni and VCAM-1, TNF-α, IL-10, and IL-6. **Figures 1,**

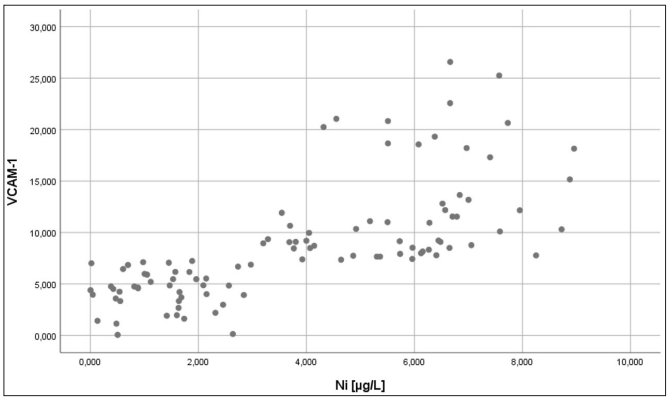


**Figure 1.** The scatter plot of the correlation between nickel (Ni) and VCAM-1 levels.

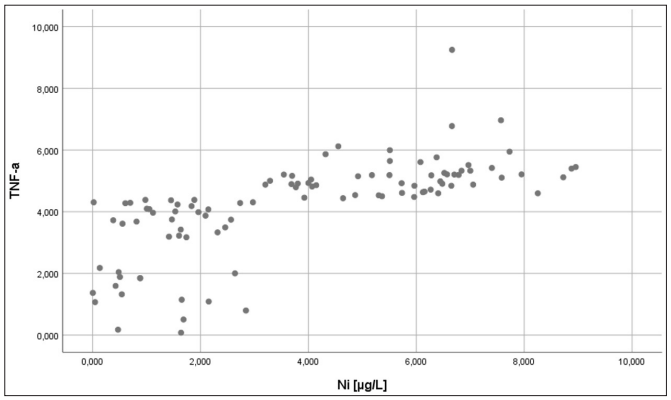


**Figure 2.** The scatter plot of the correlation between nickel (Ni) and TNF-α levels.

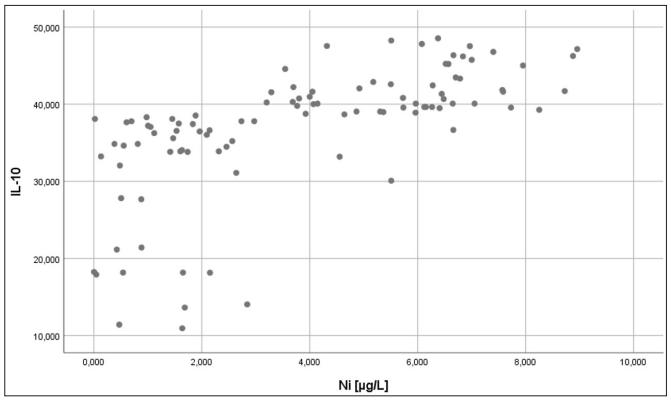


**Figure 3.** The scatter plot of the correlation between nickel (Ni) and IL-10 levels.

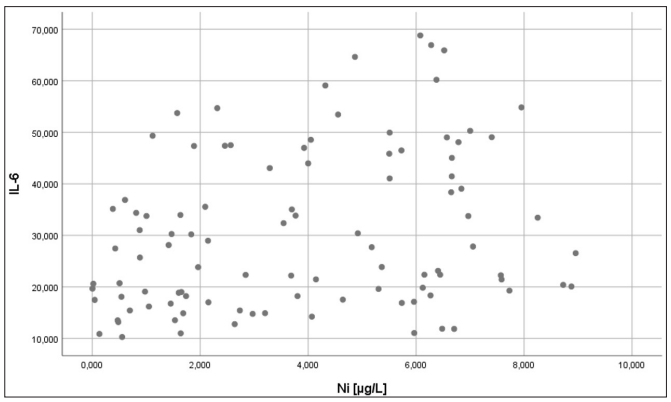


**Figure 4.** The scatter plot of the correlation between nickel (Ni) and IL-6 levels.

**2, 3**, and **4** shows the relationships between VCAM-1, TNF-α, IL-10 and IL-6 with corresponding graphs, respectively.

Among them, the strongest association was found between Ni and VCAM-1. In tumor promotion and progression, TNF-α, IL-1 and IL-6 have an important role as pro-inflammatory mediators (20). On the other hand, VCAM-1 adhesion molecule is known to induce tumor cells (21). The changes in the biomarker levels we used in our study are compatible with the studies in the literature (8,10,15) and it is one of the rare studies conducted among workers exposed to nickel. The results obtained allow these biomarkers to be used to detect both inflammation and cancer risk in annual periodic follow-ups of workers exposed to nickel. From this point of view, they are promising biomarkers in routine use.

## CONCLUSIONS

The correlations between increased inflammatory biomarkers levels and exposed/control groups propose a close relationship between inflammation and toxicity. This relationship provides a clinical model for the early diagnosis of toxicity of nickel. VCAM-1, TNF-a, IL-10 and IL-6 are promising biomarkers in routine use.

## ETHICAL DECLARATIONS

**Ethics Committee Approval:** This study was conducted with the approval of Bozok University Ethics Committee (Date: 2.10.2016; Decision No: 69).

**Referee Evaluation Process**: Externally peer-reviewed.

**Conflict of Interest Statement:** The authors have no conflicts of interest to declare.

**Financial Disclosure:** No financial support.

**Author Contributions:** All of the authors declare that they have all participated in the design, execution, and analysis of the paper and approved the final version.

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