Mechanism of Nickel and Chromium-Induced Immunotoxicity and Oxidative Stress: A Comparative Study

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Abstract

Introduction: Chromium (Cr) and Nickel (Ni) are examples of commonly used industrial substances with negative long time exposure on human health. One mechanism whereby metals can alter health is through modulation of immune homeostasis. They are capable of producing oxidative stress and it is possible that this oxidative stress contributes to the carcinogenic response of these metals.

Aim of the Work: The aim of this study is to highlight the possible immunotoxic potential of both Cr and Ni in occupationally exposed workers, to investigate the oxidative stress and their potential effects on glutathione peroxidase (GPx) among workers exposed to both metals during electroplating process.

Material and methods: The study was carried out in a hot oven factory in electroplating sector in 6th October city. The studied population included randomly selected 28 workers exposed to chromium, 12 workers exposed tonickel and 20 workers not exposed to any metal as controls. Thorough present and past history were taken. Full clinical examination was performed. Both groups were matched as regards the age and smoking habits.

Results: All workers were males with age ranging from 19 to 44 years with a mean value of 29.95 ±7.25. The duration of exposure of workers ranged from 1 to 15 years with a mean of 6.05±4.06 years. Both Ni and Cr-exposed workers had statistically significant high levels of tumor necrosis factor (TNF-α) and IL-6 and in terleukin-6 (IL-6). Statistically significant differences were found in CD3 and CD4 levels but with higher CD8 level. As regards the oxidative stress markers, serum malondialdehyde (MDA) was significantly higher in exposed groups when compared to the control group; while the serum glutathione peroxidase (GPx) was significantly lower in exposed groups.

Conclusion and Recommendations: Our study shows that chromium and nickel cause significant deviations in various immune parameters with evidence of oxidative stress in occupationally exposed workers. Medical surveillance in

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combination with biological monitoring can help to protect the workers' health.

Key Words: *Cr – Ni – Immunomodulation – Oxidative stress – Biological monitoring.*

Introduction

A LARGE number of active biological substances, including heavy metals, may have direct, primary or secondary effect on immune system. These effects can be observed as change in vivo responses, to the activities in specific immune system, as well as to the functioning of cell population. Heavy metals are of significant importance in altering the immune response by immunostimulatory or immunosuppressive mechanism. Some of them can give rise to disordered function of the immune system resulting in increased susceptibility to infection [1].

Heavy metals are ubiquitously present in the environment. Human exposure may result from sources in the industrial setting. Rapid industrialization and unchecked influent discharge and emission have alarmingly increased the contamination of various heavy metals like lead, mercury, chromium, manganese, aluminum, cadmium etc. in the environment. Exposure to some of these metals to humans is of a great concern due to the potential toxicity on the biological system [2].

One mechanism whereby metals can alter health is through modulation of immune homeostasis. Imbalances in immune regulation by metals can lead to inadequate or excessive production of inflammatory cytokines [3].

Electroplating is the process of oxidation of metal articles by the use of electrolytes containing

acids or bases. The process of electroplating involves three steps: Cleaning, plating and post-treatment of articles. Chromium is used as chromic acid, in electroplating of different articles. The workers engaged in this process are exposed to chromium through inhalation, ingestion and dermal contact. Inhalation is the primary route of occupational exposure to metals [4].

Toxicity of Cr has been demonstrated for various human cells, such as gastric mucosa cells, peripheral blood lymphocytes [5]. The major cellular targets of Cr toxicity are lung epithelial cells and lung fibroblasts, however human Cr intoxication is also associated with hepatotoxicity, nephrotoxicity, cardiotoxicity and immunotoxicity [6].

Chromium exposure has been shown to upset the immunoregulatory balance between Th1 and Th2 cells that control different immune effect or functions through the production of distinct cytokines. Chromium differentially modifies cytokine production in vitro and in vivo, preferentially activates Th2 responses [7]. A Th1 and Th2 imbalance has been implicated in the development of autoimmune disease, allergic disease, susceptibility to infectious disease, progression of AIDS, and spontaneous abortions, as well as in the aging process [8]. Immunomodulatory effects had also been demonstrated by Burastero and colleagues [9] in chromium exposed workers.

Th2 cells secrete cytokines such as IL-4, IL-5, IL-6 and IL-13, whereas Th1 cells secretes IL-2, IFN-γ TNF-α and lymphotoxin which enhance antigen presenting cell (APC) activation and the clearance of many intracellular pathogens [2]. These cytokines aid in antibody class switching and elimination of many blood born infectious agents [10].

Studies relating to occupational exposure to chromium during chromium plating process had reported nasal dysfunction, chromosome abnormality, oxidative injury to DNA, immunological effects and renal tubular dysfunction [11-13]. Several studies have reported increased levels of lipid peroxidation in plasma, blood and urine samples of workers exposed to chromium during chromium plating process [11,14].

Occupational exposure to nickel compounds occurs principally through mining, smelting, and refining operations, alloy production, electroplating and welding operations during manufacture of steel, other alloys, and batteries [15].

Besides being an allergen, nickel has been shown to exhibit immunomodulatory, if not immunotoxicity, effects in several experiments conducted in humans and in rodents. It is linked to a decrease in the number of T lymphocytes in humans [16]. Immunosuppression due to long-term exposure to nickel has been observed by Smith- Sivertsen and colleagues [17].

Exposure to nickel may lead to various adverse health effects, such as nickel allergy and contact dermatitis, organ system-toxicity as well as carcinogenesis [18-20].

The carcinogenic actions of nickel compounds are thought to involve oxidative stress, genomic DNA damage, epigenetic effects, and the regulation of gene expression by activation of certain transcription factors related to corresponding signal transduction pathways [21].

Cr and Ni are capable of producing oxidative stress and it is possible that this oxidative stress contributes to the carcinogenic response of these metals. Previous studies have demonstrated that the most potent carcinogenic forms of Ni are the water-insoluble Ni compounds such as crystalline Ni sulfide, subsulfide, and Ni oxide while the water-soluble Ni compounds have been shown to lack carcinogenic activity in vivo [22].

Costa and colleagues [23] stated that vitamin E being able to suppress a number of the genotoxic effects of Ni and Cr indicates that these metals have a substantial component of oxidative stress in the mediation of their genotoxic effects in cells. However, a portion of the Ni- and Cr-induced effects were not inhibited by vitamin E suggesting that not all effects were mediated by oxidative stress.

Aim of the work:

The aim of the study is to highlight the possible immunotoxic potential of both nickel and chromium in occupationally exposed workers, to investigate the oxidative stress and the generation of free radicals involved in plasma lipid peroxidation and their potential effects on one of the erythrocyte antioxidant enzymes (glutathione peroxidase) in workers exposed to both metals during electroplating process. Also, to investigate the relationships between malondialdehyde (MDA) as marker of oxidative stress and metal concentration in plasma, and thus to determine whether the level of MDA and glutathione peroxidaes (GPx) are related directly to Cr and Ni concentrations in plasma. Duration of exposure was also considered.

Subjects and Methods

This study was carried out in hot oven factory in electroplating sector in 6 th October city. The study population included randomly selected 28 workers exposed to chromium, 12 workers exposed to Nickel and 20 workers not exposed to any metal as control.

The control group matched the exposed group in age, sex and special habits.

An oral consent was obtained from the included workers.

Thorough present and past history were taken, about general diseases that may affect the immune system functions e.g. diabetes mellitus, auto-immune diseases, recurrent infections whether viral, bacterial or fungal infections and history of receiving immunosuppressive agents or cancer chemotherapy. Subjects having one or more of these criteria were excluded from the study.

Full clinical examination was performed with special emphasis on the skin, eye and mucous membrane infections in addition to the oral cavity and tonsils together with other body lymph nodes.

A- Sample collection:

   a- Blood samples were collected on EDTA for the estimation of the CD3, CD4, CD8 and oxidative stress markers (MDA & GPX).

   b- Serum samples were collected and stored at 80°C till TNF-α, IL6, nickel and chromium estimation.

B- Methodology:

   1- Estiamtion of CD3, CD4 and CD8 were done by flow-cytometric analysis according to Roitt and colleagues [24] using EP-ICSx LPA. Leucocyte Preparation Antigen Coulter Electronics (France).

Biosource Catalogue Nr of CD3: A07746.
Biosource Catalogue Nr of CD4: AHS0418.
Biosource Catalogue Nr of CD8: AHS0419.

The same surface markers were expressed as percentage of the total white blood cell count.

   2- Estimation of tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) levels.

TNF-α and IL6 were done by enzyme immunoassay (sandwich technique) (ELISA).

The kit was provided by immune Tech, Beckman Coulter, France.

   3- Estimation of oxidative stress markers.

Measurement of MDA:

Lipid peroxidative products were measured using the thiobarbituric acid (TBA) test for malondialdehyde (MDA), an end product of lipid peroxidation in biological tissues as described by Satoh [25] briefly, 1.0ml of the supernatant was mixed with 2.0ml of 7.5% trichloroacetic acid (TCA), then centrifuged at 1000 g for 10min. A 0.2ml of the formed supernatant was added to 0.7% thiobarbituric acid in a boiling water-bath for 15 min. The absorbance of the formed solution was determined at 532nm and a solution of 1,1,3,3- tetraethoxypropane was used as standard.

Measurement of GPx:

GPx activity was measured using glutathione peroxidase assay kit provided by Cayman Chemical Company, USA. Glutathione peroxidase assay kit measures GPx activity indirectly by a coupled reaction with glutathione reductase (GR). Oxidized glutathione (GSSG), produced upon reduction of hydroperoxide by GPx, and is recycled to its reduced state by GR and NADPH. Oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340nm. Under conditions in which the GPx activity is rate limiting, the rate of decrease in the A340 is directly proportional to the GPx activity in the sample [26].

   4- Estimation of Nickel and Chromium level.

The serum chromium and nickel level were measured by graphite furnace atomic absorption spectrophotometer with Zeeman background correction (Thermo elemental M6) [27].

1- Calibration solution preparation:

Calibrators for chromium and nickel were prepared by dilution of parent stocks which contain 1000 g/ml in each standard using deionized water.

2- Optimization technique:

For determination of metal concentrations in both standard solutions and samples, we choose proper wave length and lamp current band for each metal.

3- Calculation of the results:

The computer unit of the graphite furnace plotted a curve, the reading of absorbance of deionized water and two standard solutions for each metal

were plotted in a curve, the absorbance of the samples were blotted on these curves and the concentration of the metals in the samples were interpreted from these curves.

Data handling and statistical analysis:

Data were collected, checked, revised and entered the computer. Data were analyzed by SPSS statistical package version 17. Excel computer program was used to tabulate the results, and represent it graphically.

Qualitative variables were expressed as count and percentages.

The significant difference in distribution were tested by using Chi-square-test at $p<0.05$.

The significant difference between each two groups were tested by using independent t-test at $p<0.05$.

The significant difference between groups were tested by using One Way ANOVA followed by Duncan multiple comparison test at $p<0.05$.

Pearson correlation coefficient was calculated to show the power and direction of the linear relationship between the measured quantitative variables at $p<0.05$ [28].

Results

The studied group comprised 60 subjects divided into exposed and control groups. The workers were all males with age ranging from 19 to 44 years with a mean value of 29.95 ± 7.25. The age of the control group ranged from 21 to 40 years with a mean of 30.80 ± 6.78. There was no statistically significant difference between the exposed and control groups as regards the age and smoking habits. The duration of exposure of workers ranged from 1 to 15 years with a mean of 6.05 ± 4.06 years.

Twenty subjects among both exposed groups (50%) were cigarette smokers versus 16 subjects (80%) of the control group. Twenty subjects (50%) of the exposed group had duration of employment less than 5 years with a mean value of (2.80 ± 1.20) while 8 subjects (20%) had been employed between 5 to less than 10 years with a mean value of (6.00 ± 1.31), and 12 subjects (30%) had been employed for more than 10 years with a mean value of (11.50 ± 1.78) (Table 1).

As regards metal levels in the sera of patients; the mean value $\pm$ standard error of chromium and

nickel were 14.04 ± 2.83 µg/L and 43.78 ± 9.04 µg/L respectively, while among control group were 0.84 ± 0.33 µg/L and 1.18 ± 0.48 µg/L for Cr and Ni respectively; showing a statistically significant higher metal levels in both Ni and Cr-exposed workers as compared to controls (for Cr $p<0.01$; for Ni $p<0.001$); putting into consideration that the normal level of these metals in the serum is less than 0.5 µg/L [29].

Table (1): Demographic characteristics of the studied group.

	No.	Range Min.	Range Max.	Mean ±S.D	p-value
Age:					
Exposed Workers	40	19	44	29.95±7.25	0.760
Control	20	21	40	30.80±6.78	
Duration of exposure:					
<5 years	20	1	4	2.80±1.20	
5 – <10 years	8	5	8	6.00±1.31	0.000*
> =10 years	12	10	15	11.50±1.78	
Smoking:					
Exposed Workers	40	0	4	1.05±1.28	0.140
Control	20	0	4	1.80±1.32	

All values are represented as Mean ±S.D.

* = There is a significant difference in duration of exposure by using One Way ANOVA test at $p<0.05$.

All studied group are males, Both groups were matched as regards age and smoking.

As regards levels of proinflammatory cytokines; both Ni and Cr-exposed workers had statistically significant higher levels of tumor necrosis factor (TNF-α) (120.80 ± 15.84 and 129.20 ± 25.16 pg/dl respectively) than the control group (80.84 ± 13.55 pg/dl) ($p<0.05$). Also both groups show higher levels of interleukin IL-6 (525.10 ± 39.53 and 504.82 ± 86.06 ng/ml respectively) compared to the control group (305.59 ± 75.12 ng/ml) ($p< 0.05$) (Table 2, Fig. 1).
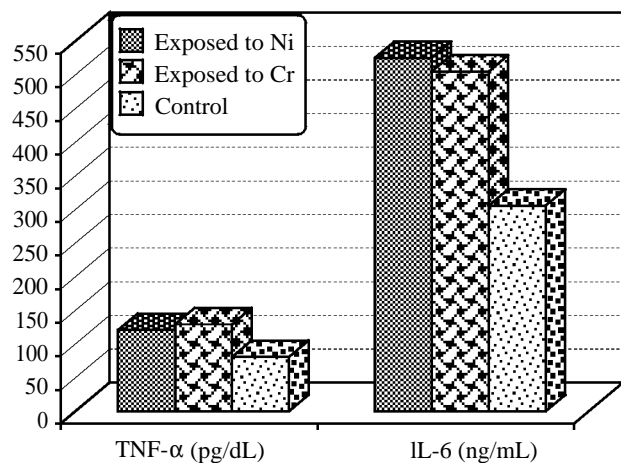

Fig. (1): Comparison between exposed workers and control group in TNF-α & IL-6.

Table (2): Comparison between exposed workers and control group in all parameters

Investigations	Exposed to Ni Mean±S.D	Exposed to Cr Mean±S.D	Control Mean±S.D	*p*-value	
TNF- α (pg/dL)	120.80 ± 15.84*	129.20 ± 25.16*	80.84 ± 13.55	<0.05	Significant
IL-6 (ng/mL)	525.10 ± 39.53*	504.82 ± 86.06*	305.59 ± 75.12	<0.05	Significant
MDA (μnol/L)	1.47 ± 0.46*	1.39 ± 0.60*	0.50 ± 0.31	<0.05	Significant
GPX (μ/gm Hb)	6.75 ± 0.64*	6.71 ± 1.49*	11.32 ± 1.36	<0.05	Significant
CD3	67.90 ± 3.45*	64.93 ± 18.48*	75.80 ± 3.08	<0.05	Significant
CD4	36.53 ± 4.77*	31.90 ± 10.32*	49.92 ± 2.64	<0.05	Significant
CD8	29.53 ± 7.33	28.33 ± 9.72	24.99 ± 2.26	>0.05	No Significance
CD4/CD8	1.32 ± 0.42	1.25 ± 0.46	1.98 ± 0.14	>0.05	No Significance

All values are expressed as Mean ± Standard Deviation.
The significant difference between groups were done by using One way ANOVA test at *p*<0.05.
* = There is a significant difference about control group by using Dunnete *t*-test at *p*<0.05.
TNF-α: Tumour necrosis factor.
IL-6 : Interleukin-6.
MDA: Malon dial dehyde.
GPx : Glutathione peroxidase.
CD : Cluster determinant.

As regards the immune parameters, CD levels were measured as markers of T helper cells, cluster determinant CD3, CD4 and CD8 were measured, the mean CD3 levels were lower in both exposed groups (67.90±3.45 in Ni group and 64.93 ± 18.48 in Cr group) when compared with control group (75.80±3.08), with statistically significant difference (*p*<0.05). Also there is a statistically significant lower CD4 levels in both Ni and Cr exposed groups (36.53±4.77 and 31.907± 10.32 respectively) than the control group (49.92±2.64) (*p*<0.05). CD 8 levels were higher in both exposed groups (29.53±7.33 in Ni group and 28.33±9.72 in Cr group) than in control group (24.99 ±2.26), but this difference did not reach level of significance (*p*>0.05).

Both exposed groups had lower CD4/CD8 levels (1.32±0.42 in Ni group and 1.25 ±0.46 in Cr group) than the control group (1.98 ±0.14) but with no statistically significant difference (*p*>0.05) (Table 2, Fig. 3).

As regards the oxidative stress markers, serum malondialdehyde (MDA) was significantly higher in exposed groups (1.47±0.46 in Ni group and 1.39±0.6 in Cr group) when compared to the control group (0.50±0.31); while the serum glutathione peroxidase (GPx) was significantly lower in exposed groups (6.75±0.64 in Ni group and 6.71 ±1.49 in Cr group) compared to control group (11.32±1.36) (*p*<0.05) (Table 2, Fig. 2).
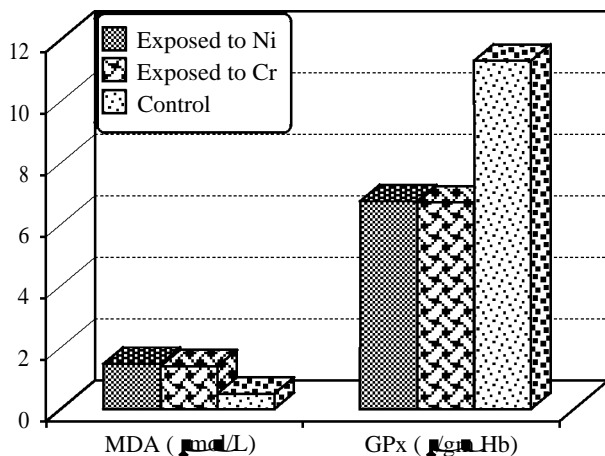

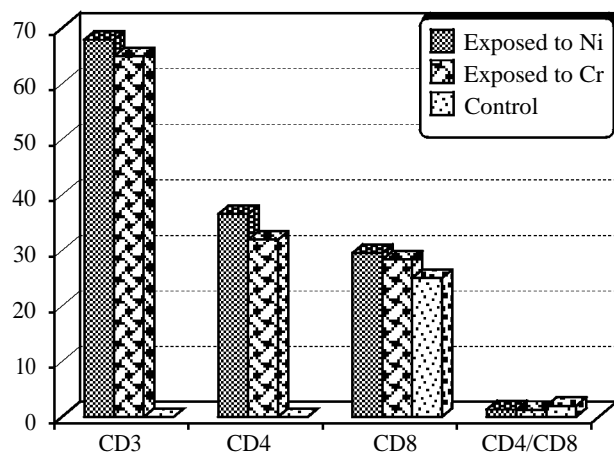Fig. (2): Comparison between exposed workers and control group in MDA & GPX.

Fig. (3): Comparison between exposed workers and control group in CDs.

As regards effect of the duration of exposure; in Ni group there was a statistically significant difference between the duration of exposure and most of the investigated parameters; in GPx level (*p*<0.001), CD3 level (*p*<0.01), CD8 (*p*<0.01) and C4/CD8 ratio (*p*<0.01) (Table 3).

In chromium group, there was a statistically significant difference between the duration of exposure and most of the investigated parameters; in IL6 level (*p*<0.01), CD3 (*p*<0.01), CD8 (*p*<0.01) and CD4/CD8 ratio (*p*<0.01) (Table 4).

Table (3): Effect of duration of exposure to Ni on the studied variables .

Investigations	<5 years	5 – <10 years	> =10 years	F- value	*p*-value
	Mean ±S.D	Mean ±S.D	Mean ±S.D		
TNF- α (pg/dL)	125.95 ± 1.67	124.90 ± 17.45	98.20 ± 0.10	3.61	0.071
IL-6 (ng/mL)	546.85 ± 62.99	521.43 ± 15.52	492.60 ± 20.00	1.40	0.295
MDA (μmol/L)	1.74 ± 0.21	1.38 ± 0.59	1.20 ± 0.01	1.17	0.353
GPx (/gm Hb)	7.33 ± 0.26	6.30 ± 0.02	5.92 ± 0.13	54.05	0.000*
CD3	70.57b ± 1.60	65.05a ± 3.29	65.60a ± 1.25	8.51	0.008 *
CD4	36.43 ± 2.42	38.50 ± 7.74	32.90 ± 3.15	0.90	0.439
CD8	30.90b ± 6.49	22.75 a ± 1.33	39.00b ± 5.20	7.81	0.011 *
CD4/CD8	1.27b ± 0.31	1.65b ± 0.40	0.80a ± 0.01	4.55	0.043 *

All values are expressed as Mean ± Standard Deviation
* = There is a significant difference between groups by using One Way ANOVA at *p*<0.05
The same letter means that no significant difference by using Duncan multiple comparison test at *p*<0.05

Table (4): Effect of duration of exposure to Cr on the studied variables.

Investigations	< 5 years	5 - <10 years	> =10 years	F- value	*p*-value
	Mean ±S.D	Mean ±S.D	Mean ±S.D		
TNF- α (pg/dL)	138.15 ± 36.89	131.70 ± 29.40	122.12 ± 9.77	0.70	0.505
IL-6 (ng/mL)	602.95b ± 11.83	501.50a ± 68.326	479.15a ± 91.85	3.93	0.033 *
MDA (μmol/L)	1.16 ± 0.24	1.39 ± 0.21	1.48 ± 0.99	0.38	0.689
GPx (/gm Hb)	7.12 ± 1.17	6.70 ± 1.52	6.57 ± 1.67	0.18	0.834
CD3	72.30b ± 5.70	66.90ab ± 9.46	53.84a ± 26.93	3.47	0.047*
CD4	36.07 ± 3.95	24.10 ± 3.69	29.20 ± 15.18	3.02	0.067
CD8	30.59b ± 5.91	36.25b ± 6.06	22.00a ± 11.86	4.93	0.016*
CD4/CD8	1.22b ± 0.35	0.70a ± 0.01	1.52b ± 0.50	6.19	0.007*

All values are expressed as Mean ± Standard Deviation
* = there is a significant difference between groups by using One Way ANOVA at *p*<0.05
The same letter means that no significant difference by using Duncan multiple comparison test at *p*<0.05

Table (5) shows comparison between the exposed and control groups as regards clinical manifestations; among Ni group, about 33.3% of workers were suffering from chest wheezes while only in 30% of control group, also 42.1% of workers exposed to chromium were suffering from the same complaint. Among workers exposed to nickel, 66.7%, 66.7% and 16.7% were suffering from epistaxis, skin problems and infection of mouth respectively. Among workers exposed to chromium 57.9%, 26.3% and 36.8% were suffering from epistaxis, skin problems and infection of mouth respectively.

There was a negative correlation between the serum Ni level and the MDA level (*r*=-0.597, *p*<0.041), also with the serum GPx level (*r*=-0.438, *p*<0.154). Moreover, there was a positive correlation between the serum Cr and the CD8 level (*r*=0.448, *p*<0.017) and a negative correlation with CD4/CD8 ratio (*r*=-0.446, *p*<0.017) (Table 6).

Table (5): Comparison between exposed workers and control group regarding clinical manifestations.

Clinical manifestations	Exposed to Ni (n=12)		Exposed to Cr (n=28)		Control (n=20)		χ^2	p-value
	No.	%	No.	%	No.	%		
Chest wheezes	4	33.3%	16	42.1%	3	30.0%	13.65	0.001*
Epistaxis	8	66.7%	22	57.9%	–	–	–	–
Skin problems	8	66.7%	10	26.3%	–	–	–	–
Infection of mouth	2	16.7%	14	36.8%	–	–	–	–

* = A significant difference between exposed and control as regards chest wheezes, by using Chi-square test at $p<0.05$.

Table (6): Correlation between the studied variables within exposed group.

	Ni ($\mu g/L$)	Cr ($\mu g/L$)
MDA ($\mu mol/L$)		
r	– 0.597	– 0.16
p-value	0.041	0.415
N	12	28
GPX ($\mu g/gm\,Hb$)		
r	– 0.438	– 0.015
p-value	0.154	0.939
N	12	28
CD8		
r	0.398	0.448
p-value	0.2	0.017
N	12	28
CD4/CD8		
r	– 0.322	– 0.446
p-value	0.307	0.017
N	12	28

Correlation is significant at the 0.05 level

Discussion

Immunotoxicity is among the numerous health effects of heavy metal exposure. The most immunosuppressive of the heavy metals was found to be mercury based on the results of animal experiments. Copper, manganese, cobalt, cadmium, and chromium in decreasing potential for immunosuppression, share the same properties [30].

The current study shows that all exposed workers had significantly higher blood chromium and nickel levels as compared to the control group.

The toxicity of chromium at high and moderate doses has been known for long, toxicity at lower doses has received perhaps more toxicological and epidemiological attention than any other reagent. Because Th1 and Th2 cells are crucial regulators of immune responses, proper differentiation of naïve CD4+ Th cells into Th1 or Th2 cells is critical for T-dependent immune responses [31]. In human

Th 1 cytokine profile (TNF-α, IFN-γ) is essential for protection against intracellular pathogens while a Th 2 profile (IL-4, IL-5, IL-6) is associated with diseases characterized by overproduction of antibodies including Ig E [1].

At low levels, cytokines are essential for optimal functioning of our defense and repair system. Cytokine regulation is also required to control excessive effector T-cell responses against exogenous antigens, when they become dangerous for the body [32].

In our study, workers exposed to chromium and nickel had statistically significant lower CD3, CD4 levels with non-significant lower CD4/CD8 ratio and non-significant higher CD8 levels than the control group. This finding can be explained by the fact that CD8+ T cells are known to mediate delayed type hypersensitivity (DTH) responses in allergic contact dermatitis, drug eruptions, asthma, and autoimmune diseases [33]. The presence of CD4+ T cells has a negative effect on the intensity of the CD8+ T cell-mediated contact hypersensitivity (CHS) response [34,35]. So, a functional dichotomy exists between CD8+ T cells and CD4+ T cells which behave as effector cells and regulatory cells, respectively in CHS [36]. So, it is not uncommon to find low CD4 and high CD8 levels.

Similarly, as regards Cr exposure, a statistically significant negative association was found between chromium urine concentration and the total T cell count, CD4 cell count and other CDs memory T helper cells on the epidemiological study carried by Kamaus and colleagues [37] that focused on heavy metals exposure in a toxic waste incineration plant. Also, the study of Boscolo and colleagues [38] who examined a group of 15 workers exposed to lead chromate dust by inhalation at work and who had raised blood and urine chromium levels, had reduced levels of circulating CD4+ helper, activated B and natural killer cells.

On the other hand, Hanovcova and colleagues [39] found higher counts of T-lymphocytes, CD4 and CD8 lymphocytes in their examined group of workers.

As regards, Ni group, the study of Minang and colleagues [40] demonstrated that Ni suppress the Ni-specific Th1-type CD4+ T cells.

Also, analyses of Ni-specific T-cell clones generated from h-PBMC and skin of allergic patients have suggested that both CD4+ and CD8+ T cells are involved in the immune response to Ni [41,42].

The study of Boscolo and colleagues [43] evaluated the immune response to ingestion of 10mg of nickel (as Ni sulphate) in 19 young non-atopic Ni-sensitized and 9 non-allergic women (as control group). Twenty-four hours following Ni ingestion, the "non-responder" group showed no modification of lymphocyte subsets in relation to the basal values; however, the "responder" group (who showed allergic symptoms) showed a significant reduction of circulating total blood lymphocytes and particularly of CD3+, CD4+ and CD8+ suggesting that Ni absorption is higher in "responder" patients indicating change of immune response. The marked reduction of blood T lymphocytes of symptomatic (responder) patients after Ni challenge seems to depend on migration of these cells in the tissues (including gastrointestinal mucosa). At the same time, Boscolo and colleagues found low CD4 and high CD8 levels in the control group and found it difficult to be explained since it is known that Ni binds to class II proteins of the major histocompatibility complex presenting cells to CD4+ lymphocytes and seems not to have direct effects on CD8+ lymphocytes. These data point to the possibility of alteration of CDs levels according to the degree of absorption, sensitization, susceptibility, dose and duration of exposure in workers.

Both Cr and Ni groups had statistically significant higher TNF-α and IL6 levels than the control group indicating immunomodulation and initial activation of Th1 and Th2 responses characterized by increased level of TNF-α and of IL-6 (Table 2) which was evident in workers up to 10 years of exposure; after which gradual decrease in both levels occurred indicating immunosuppression and failure of the immune system to cope with continued exposure (Table 3 & 4).

Similar results were previously explained by Kuo and Wu [12] who found that exposure to low doses of Cr in the early stages can stimulate IL-6

and IL-8, thereby increasing levels of these immunological parameters. Also, Glaser and colleagues [44] had stated that respiratory defense and immunological functions were stimulated or inhibited depending on dose and time of Cr inhalation. Villanueva and colleagues [7] also reported that chromium preferentially activates Th2 responses, which are characterized by IL-6 and to lesser extent, IL-5, IL-10 and IL-13 secretion and promote humoral immunity. Also, Granchi and colleagues [45] found increased levels of TNF-α and IL6 on examination of PBMC of their patients. As, Dayan and Paine [46] who found increased level of TNF-α in vitro studies on fresh human keratinocytes.

On the other hand, Katiyar and colleagues [1] had proven that there was no difference in TNF-α level in the sera of chromium exposed individuals; but IL-6 levels were suppressed in exposed groups as compared to unexposed healthy volunteers which is in conformity with earlier studies [47-49]. Out of these, some studies were conducted on cell cultures and some on animals. The studies on animals [50] regarded as more comprehensive as hormonal and nervous control are found intact. Cell culture studies are confined to a particular cell and therefore effectively explain the local production of cytokines. In both types of studies the IL-6 levels decrease proportionally with increased chromium level in human peripheral blood mononuclear cells (h-PBMCs). A major effect of IL-6 is to act on the hypothalamus, altering the body's temperature regulation and on muscle and fat cells altering energy mobilization to increase the body temperature. At elevated temperatures, bacterial and viral replications are decreased, while processing of antigen is enhanced leading to increase the adaptive immune response.

Also, Snyder and colleagues [51] investigated IL-6 levels among individuals in Hudson County, New Jersey (an area contaminated with Cr) and found that IL-6 levels were significantly lower than those in non-contaminated areas; however, they studied an area contaminated with Cr but did not report on individual levels of urine or serum-Cr.

In Ni group, Ni level although higher than the control group but not reaching the level of provoking immunosuppression as evidenced by higher levels of TNF-α and IL6 levels up to 10 years of exposure. Also, Taira and colleagues [52] proved that Ni ions dose dependently increased release of three inflammatory cytokines (IL-6, IL-1 β and TNF-α) and oxidative stress conditions in murine macrophage-like cells.

On the contrary Harkin and colleagues [53] found that NiCl2 inhibited production of the pro-inflammatory cytokine TNF- a and increased production of the anti-inflammatory cytokine IL-10 from lipopolysaccharide (LPS) stimulated cultures as NiCl2 suppresses T-cell function and promotes an immunosuppressive macrophage phenotype in rats upon exposure to very high concentration of Ni.

In our study, progressive decrease in TNF- a and IL-6 levels occurred with increased duration of exposure. This can be explained by the study of Pascal and Tessier [54] who stated that the toxicity of chromium can result in the initiation of intracellular signaling processes, the production of inflammatory mediators and through these mediators, a subsequent involvement of the immune system. This is associated with IL-6, IL-8 and TNF- a release. The dose-response relationships for both IL-6 and IL-8 release following chromium exposure was inverted-U shaped. Therefore, the maximum cytokine release observed occurred not at the highest dose of the test metal, but at that concentration where the cytotoxic loss of cells capable of producing and releasing the respective cytokines did not impact the overall media concentrations of those cytokines. At the highest metal concentrations, then, cytotoxicity substantially reduced the number of cytokine releasing cells resulting in reduced cytokine concentrations. This finding could be applied to repeated or chronic exposure. So, with increased duration of exposure cytokine release was decreased making the exposed workers more prone to several bacterial and viral infections.

The exposure of cells to environmental oxidants such as UV and ionizing radiation, heavy metals, redox active chemicals, hypoxia and hyperoxia increases reactive oxygen species (ROS) production which shifts cell redox status to a more oxidized state known as an oxidative stress. ROS are capable of causing direct damage effect or acting as critical intermediate signaling molecules leading to diverse biological consequences [55-57]. Toxic stress resistance or adaptation of cells to shift in cell redox status could be characterized by the response of the intracellular antioxidant defense system [58].

Erythrocytes have been indicated as the circulating antioxidant involving several enzymes-glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT)- and levels of their antioxidant factors could reflect the degree of oxidative stress [59,60].

Lipoperoxidation occurs as a chain reaction initiated by free radicals which propagates itself and can result in the formation of many equivalents of lipid peroxides; the extension of the oxidative catabolism of lipid membranes can be evaluated by the quantification of malondialdehyde (MDA) one of the stable aldehydic products of lipoperoxidation present in biological samples as whole blood, plasma or urine [61].

The toxicity and carcinogenicity of Ni compounds in humans and animals have been well documented [62,63]. One possible mode by which Ni causes cell death and/or damage may involve oxidative reaction such as lipid peroxidation (LPO) [63,64]. The production of reactive oxygen species (ROS) is involved in the molecular mechanisms of Ni toxicity and carcinogenicity [21,65]; that in turn can lead to enhanced formation of MDA in the cells [66].

In our study both Cr and Ni exposed workers had statistically significant higher MDA levels than the control group which indicate an increase in generation of ROS and lipid peroxidation. This level increased with the duration of exposure in Cr group (Table 4) carrying the risk of possible genotoxicity and carcinogenesity in exposed workers. However, in Ni group the level of MDA decreased with the duration of exposure (Table 3) indicating oxidative stress not damage.

Similarly, the study done by Imamoglu and colleagues [67] to study the erythrocyte antioxidant system (superoxide dismutase and catalase) and lipid peroxidation (malondialdehyde) in the erythrocyte membrane of workers continously exposed to welding fumes and gases, They found as in our study that the range of MDA levels in the welder subjects was much higher than in controls. However, the difference was not statistically significant. In contrast, they found no correlation between the MDA level and the metal concentrations in plasma of exposed workers regarding the duration of exposure in all welders. They attributed this finding to the oxidative stress triggered by continuous exposure to welding fumes, but it was probably well balanced with the antioxidant defense system since the erythrocyte lipids were not peroxidated; which is not the case in our study as the oxidative stress was not compensated by the antioxidant defense system as evidenced by the high level of MDA which is positively correlated with the duration of exposure (Table 6) suggesting the probable occurrence of oxidative damage not only stress. Also El Samra and colleagues in 2007 [68] as well as Bagchi and colleagues in 2002 [69] found increased formation of ROS and lipid peroxidation in Cr exposure.

Similarly the study of Kalahasthi and colleagues [70] noted that plasma lipid peroxidation was significantly increased and the levels of antioxidant enzymes were significantly decreased in chromium-exposed subjects. The level of plasma lipid peroxidation was positively and levels of antioxidant enzymes were negatively and significantly correlated with the levels of chromium in urine, making them useful as biomarkers of oxidative stress that would help in early detection of high-risk subjects.

Also the study of Kuo and colleagues [71] showed an increase of urinary 8 - hydroxydeoxyguanosine (a marker of oxidative stress to DNA) concentrations in electroplating workers.

In accordance with our results in Ni group, the study of Chen and colleagues [72] has demonstrated that Ni causes an increase in intracellular ROS, which may have the ability to cause oxidative damage in isolated human lymphocytes.

Although $NiCl_2$-induced increase in oxidative stress has been observed in human plasma [73] and lymphocytes [72], how such oxidative stress relates to cellular toxicity/cell death due to $NiCl_2$ is not known.

As a consequence of oxidative stress, both Ni and Cr exposed workers showed statistically significant lower GPx levels as compared to the control group which continue to decrease with increased duration of exposure; however not reaching the level of significance; this provides evidence that this antioxidant may be involved in protection of the cell against induced toxicity and that long-time exposure to very low doses of metal salts with a consequent increase in free radical production could lead to a chronic consumption of outstanding antioxidants such as GPx, thus affecting the overall antioxidant capacity.

This fact could point to the exposure to toxic levels of metals as evidenced by the study of Asatiani and colleagues [74] who stated that the nontoxic concentration of Cr did not cause an appreciable increase of catalase and GPx activities compared with corresponding controls, whereas under sub-toxic concentration increase of catalase activity corresponds to the sharp reduction of GPx activity, which becomes stronger at toxic concentration that causes complete exhaustion of GPx, while catalase still functions. They concluded that chromium affects all antioxidants in the enzymatic antioxidant pathways depending on the concentration range. In the case of a nontoxic chromium dose, temporary oxidative stress is overcome by increased activity of the antioxidant system with

correlation to cell cycle re-entry. The toxic concentrations misbalance the cell antioxidant defense systems and cause irreversible growth arrest and massive cell death by apoptosis.

Also, the study of De Mattia and colleagues [75] demonstrated that in humans, an oxidative stress occurred for Cr exposures as low as those considered safe as evidenced by decrease in GPx. This oxidative stress appears to be able to affect intracellular and plasmatic antioxidant defense.

Stepniewski and colleagues [76] found a significantly valid relationship between the Cr and total antioxidant status (TAS) levels in serum and hair samples of welders who have been working for 23.7 years.

Cr and Ni are known to bind to GPx producing stable coordinate complexes that could be excreted from cells. The presence of elevated concentrations of Cr and Ni can also result in oxidative stress, which would result in the depletion of GPx due to its role in radical scavenging. A combination of the stress-mediated depletion and the participation of GPx in binding and excretion of metals could potentially be responsible for the observed decrease in the amount of free intracellular GPx in lymphocytes from welders [77].

Similarly, Das Gupta and colleagues [78] proved that nickel and chromium increased lipid peroxide (LPO) and decreased glutathione levels, as well as the activity of superoxide dismutase (SOD), catalase, and glutathione peroxidase.

As regards clinical manifestations, (Table 5) shows that 66.7% of workers exposed to Ni and 26.3% of those exposed to Cr suffered skin problems owing to alteration in the immune response. Patients experienced pruriginous erythema-vesicular manifestations on the hands and wrists and/or eczema and pruritus on the face, neck and chest; two patients showed generalised urticaria. Similarly, the study of Boscolo and colleagues [43] showed that most of the examined Ni group suffered from allergic contact dermatitis (ACD). The study of Saint-Mezard and colleagues [36] showed that CHS reaction and ACD can be viewed as the result of activation of two distinct T cell subsets endowed with opposite functions: Effector T cells and down-regulatory T cells. The severity and the duration of the skin inflammation appear directly related to the respective activation state and/or size of these two compartments. Thus, overwhelming regulation in sensitized individuals may lead to lack of inflammation (tolerance) despite repeated exposures to the hapten, while defects in regulatory cells may

explain chronic contact dermatitis. The study of Curtis and colleagues [79] proved that both metals are capable of causing contact dermatitis but chromium was more cytotoxic than nickel.

High TNF-α and CD8 levels could result in amplifying the inflammatory response as stated by Traidl and colleagues [42]; a finding that explains increased chest and skin problems with infection of the mouth among exposed workers.

Conclusion and Recommendations:

Chromium and nickel are examples of commonly used industrial substances with negative long time exposure on human health. Our study shows that chromium and nickel cause significant deviations in various immune parameters with evidence of oxidative stress in occupationally exposed workers. However, it is necessary to undertake further research into the precise biological mechanisms by which both metals damage the immune system.

A special health surveillance program must focus on known health impairments and target organs. Medical surveillance in combination with biological monitoring can help to protect the workers' health. The industries using chromium and/or nickel should evolve some technique so as to reduce their levels of exposure through continuous engineering improvements with efficient local exhaust ventilation system.

Biological monitoring for exposure in urine, blood, and erythrocytes provides different types of information. Whereas chromium measurement in urine and whole blood or plasma is indicative of recent total chromium exposure, chromium detection in erythrocytes is attributable to Cr only and covers retrospectively a longer period of time due to the erythrocyte life span.

Continuous education of workers about the importance of proper and regular use of protective measures.

Nutritional antioxidants supplementation is recommended owing to the oxidative stress and/or damage induced by occupational exposure to both metals.

References

1- KATIYAR S., AWASTHI S.K. and SAHU R.K.: Suppression of IL-6 level in human peripheral blood mononuclear cells stimulated with PHA/LPS after occupational exposure to chromium. Sci. Total Environ., 390 (2-3): 355-61, 2008.

2- KATIYAR S., AWASTHI S.K. and SRIVASTAVA J.K.: Effect of chromium on the level of IL-12 and IFN-gamma in occupationally exposed workers. Sci. Total Environ., 407 (6): 1868-74, 2009.

3- LAWRENCE D.A. and MCCABE Jr. M.J.: Immunomodulation by metals. Int. Immunopharmacol., 2: 293-302, 2002.

4- KELLEHER P., PACHECO K. and NEWMAN L.S.: Inorganic dust pneumonias: The metal related parenchyma disorders, Environ. Health Perspect., 108 (4): 685-96, 2000.

5- TRZECIAK A., KOWALIK J., MALECKA-PANAS E., DRZEWOSKI J., WOJEWODZKA M., IWANENKO T. and BLASIAK J.: Genotoxicity of chromium in human gastric mucosa cells and peripheral blood lymphocytes evaluated by single cell gel electrophoresis (comet assay). Med. Sci. Monit., 6: 24-29, 2000.

6- POURAHMAD J. and O'BRIEN T.J.: Biological reactive intermediates that mediate Chromium (VI) toxicity. In: Dansette PM (ed) Biological reactive intermediates VI: Chemical and biological mechanisms in susceptibility to and prevention of environmental diseases: Advances in experimental medicine and biology. Springer, Berlin, p. 203, 2001.

7- VILLANUEVA M.B.G., KOIZUMI S. and JONAI H.: Cytokine production by human peripheral blood mononuclear cells after exposure to heavy metals. J. Health Sci., 46 (5): 358-62, 2000.

8- VALENTINO M., RAPISARDA V., SANTARELLI L., BRACCI M., SCORCELLETTI M. and DI LORENZO L.: Effect of lead on the levels of some immunoregulatory cytokines in occupationally exposed workers. Human Exp. Toxicol., 26: 551-6, 2007.

9- BURASTERO S.E., PAOLUCCI C. and BREDA D.: Chromium (VI)-induced immunotoxicity and intracellular accumulation in human primary dendritic cells. Int. J. Immunopathol. Pharmacol., 19 (3): 581-91, 2006.

10- ABBAS A.K., MURPHY K.M. and SHER A.: Functional diversity of helper T lymphocytes. Nature., 383: 787-93, 1996.

11- MAENG S.H., CHUNG H.W., KIM K.J., LEE B.M., SHIN Y.C., KIM S.J. and YU I.J.: Chromosome aberration and lipid peroxidation in chromium-exposed workers. Biomarkers, 9 (6): 418-34, 2004.

12- KUO H.W. and WU M.L.: Effects of chromic acid exposure on immunological parameters among electroplating workers. Int. Arch. Occup. Environ. Health, 75: 186-90, 2002.

13- LIU C.S., KUO H.W., LAI J.S. and LIN T.I.: Urinary, N-Acetyl-β-D-glucosaminidase as an indicator of renal dysfunction in electroplating workers. Int. Arch. Occup. Environ. Health, 71: 348-52, 1998.

14- HUANG Y.L., CHEN C., SHEU J.Y., CHUANG I.C., PAN J.H. and LIN T.H.: Lipid peroxidation in workers exposed to hexavalent chromium. J. Toxicol. Environ. Health Part A, 56: 235-47, 1999.

15- M'BEMBA-MEKA P., LEMIEUX N. and CHAKRABARTI S.K.: Role of oxidative stress and intracellular calcium in nickel carbonate hydroxide-induced sister-chromatid exchange, and alterations in replication index and mitotic index in cultured human peripheral blood lymphocytes. Arch. Toxicol., 81: 89-99, 2007.

16- Eggleston D.W.: Effect of dental amalgam and nickel alloys on T-lymphocytes: Preliminary report. J. Prosthet. Dent., 51: 617- 23, 1984.

17- SMITH-SIVERTSEN T., TCHACHTCHINE V. and LUND E.: Environmental nickel pollution: Does it protect against nickel allergy? J. Am. Acad. Dermatol., 46: 460-2, 2002.

18- LEACH C.N. JR., LINDEN J.V., HOPFER S.M., CRISOSTOMO M.C. and SUNDERMAN F.W. Jr.: Nickel concentrations in serum of patients with acute mycardinal infarction or unstable angina pectoris. Clin. Chem., 31: 556-60, 1985.

19- VYSKOCIL A., SENFT V., VIAU C., CIZKOVA M. and KOHOUT J.: Biochemical renal changes in workers exposed to soluble nickel compounds. Hum. Exp. Toxicol., 13: 257-61, 1994.

20- GAWKRODGER D.J., LEWIS F.W. and SHAH M.: Contact sensitivity to nickel and other metals in jewelry reactors. J. Am. Acad. Dermatol., 43: 31-6, 2000.

21- LU H., SHI X., COSTA M. and HUANG C.: Carcinogenic effect of nickel compounds. Mol. and Cell. Bioch. 279: 45-67, 2005.

22- International Agency for Research on Cancer (IARC): Chromium, nickel and welding. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 49. World Health Organization, Lyon., France. p. 677, 1990.

23- COSTA M., SALNIKOW K., SUTHERLAND J.E., BRODAY L., WU PENG W., ZHANG Q. and KLUZ T.: The role of oxidative stress in nickel and chromate genotoxicity. Mol. and Cell Bioch., 234/235: 265-75, 2002.

24- ROITT I., BROSTOFF S. and MALE D.: Immunology. 5th ed., London. Mosby, 1998.

25- SATOH K.: Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method .Clin. Chim. Acta. 90: 37-43, 1978.

26- ROXBOROUGH H.E., MERCER C., MCMASTER D., MAXWELL A.P. and YOUNG I.S.: Plasma glutathione peroxidase activity is reduced in haemodialysis patients. Nephron., 81: 278-83, 1999.

27- TAYLOR A.: Trace elements external quality assessment scheme, SAS. Trace. Element. Handbook, 1998.

28- ARMITAGE P.: G. Berry, J.N.S. Matthews (Fourth Edition): Statistical Methods in Medical Research, Blackwell Science Ltd, London, 2002.

29- LAUWYERS R. and HOET P.: Industrial chemical exposure: Guidelines for biological monitoring Bosco Raton: Lewis, 1993.

30- BERNIER J., BROUSSEAU P., KRZYSTYNIAK K., TRYPHONAS H. and FOURNIER M.: Immunotoxicology of heavy metals in relation to great lakes. Environ. Health Perspect., 103 (9): 23-34, 1995.

31- DONG C. and FLAVELL R.A.: Cell fate decision: T-helper 1 and 2 subsets in immune responses. Arthritis Res. 2 (3): 179-88, 2000.

32- ROMAGNANI S.: Regulation of the T cell response. Clin. Exp. Allergy., 36 (11): 1357-66, 2006.

33- KALISH R.S. and ASKENASE P.W.: Molecular mechanisms of CD8+ T cell-mediated delayed hypersensitivity: Implications for allergies, asthma, and autoimmunity. J. Allergy. Clin. Immunol., 103: 192-9, 1999.

34- BOUR H., PEYRON E., GAUCHERAND M., GARRIGUE J.L., DESVIGNES C., KAISERLIAN D., REVILLARD J.P. and NICOLAS J.F.: Major histocompatibility complex class I-restricted CD8+ T cells and class II-restricted CD4+ T cells, respectively, mediate and regulate contact sensitivity to dinitrofluorobenzene. Eur. J. Immunol. 25: 3006-10, 1995.

35- BOULOC A., CAVANI A., KATZ S.I., FONG T.A., MOSMANN T.R., XU H., DIIULIO N.A. and FAIRCHILD R.L.: Contact hypersensitivity in MHC class II-deficient mice depends on CD8 T lymphocytes primed by immunostimulating Langerhans cells. J. Invest. Dermatol., 111: 44-9, 1998.

36- SAINT-MEZARD P., BERARD F., DUBOIS B., KAISERLIAN D. and NICOLAS J.F.: The role of CD4+ and CD8+ T cells in contact hypersensitivity and allergic contact dermatitis. Eur. J. Dermatol., 14: 131-8, 2004.

37- KAMAUS W., HUANG S., OSIUS N. and NEBE T.: Chromium urine concentration and effects on lymphocyte subpopulations in children. J. Environ. Med., 1 (3): 153-61, 1999.

38- BOSCOLO P., DI GIOACCHINO M., BAVAZZANO P., WHITE M. and SABBIONI E.: Effects of chromium on lymphocyte subsets and immunoglobulin from normal population and exposed workers. Life Sci., 60: 1319-25, 1997.

39- HANOVCOVA I., CHYLKOVA V., TEJRAL J., ANDRYS C., PROCHAZKOVA J., TURKOVA M., SRB V., ETTLEROVA E. and KOKES J.: Long- term monitoring of the immune reactivity of stainless steel welders. Cent. Eur. J. Public Health, 6 (1): 51-6, 1998.

40- MINANG J.T., ARESTRÖM I., ZUBER B., JÖNSSON G., TROYE-BLOMBERG M. and AHLBORG N.: Nickel-induced IL-10 down-regulates Th1- but not Th2-type cytokine responses to the contact allergen nickel. Clin. Exp. Immunol., 143 (3): 494-502, 2006.

41- MOULON C., WILD D., DORMOY A. and WELTZIEN H.U.: MHC-dependent and -independent activation of human nickel-specific CD8+ cytotoxic T cells from allergic donors. J. Invest. Dermatol., 111: 360-6, 1998.

42- TRAIDL C., SEBASTIANI S., ALBANESI C., MERK H.F., PUDDU P., GIROLOMONI G. and CAVANI A.: Disparate cytotoxic activity of nickel-specific CD8+ and CD4+ T cell subsets against keratinocytes. J. Immunol., 165: 3058-64, 2000.

43- BOSCOLO P., ANDREASSI M., SABBIONI E., REALE M., CONTI P., AMERIO P. and DI GIOACCHINO M.: Systemic effects of ingested nickel on the immune system of nickel sensitized women. Life Sciences, 64 (17): 1485-91, 1999.

44- GLASER U., HOCHRAINER D., KLOPPEL H. and KUKNEN H.: Low level chromium (IV) inhalation effects on alveolar macrophage and immune functions in Wistar rats. Arch. Toxicol., 57: 280-6, 1985.

45- GRANCHI D., CIAPETTI G. and STEA S.: Cytokine release in mononuclear cells of patients with Co-Cr hip prosthesis. Biomaterials, 20: 1079-86, 1999.

46- DAYAN A.D. and PAINE A.J.: Mechanisms of chromium toxicity, carcinogenicity and allergenicity: Review of the literature from 1985 to 2000. Hum. Exp. Toxicol., 20: 439-51, 2001.

47- JAIN S.K. and LIM G.: Chromium chloride inhibits TNF-α and IL-6 secretion in isolated human blood monoclear cells exposed to high glucose. Horm. Metab. Res., 38: 60-2, 2006 a.

48- JAIN S.K., PATEL P., ROGIER K. and JAIN S.K.: Trivalent chromium inhibits protein glycosylation and lipid peroxidation in high glucose-treated erythrocytes. Antioxid. Redox. Signal., 8 (1-2): 238-41, 2006 b.

49- JAIN S.K., RAINS J.L. and CROAD J.L.: High glucose and ketosis (acetoacetate) increases, and chromium niacinate decreases, IL-6, IL-8, and MCP-1 secretion and oxidative stress in U937 monocytes. Antioxid. Redox. Signal., 9 (10): 1581-90, 2007a.

50- JAIN S.K., RAINS J.L. and CROAD J.L.: Effect of chromium niacinate and chromium picolinate supplementation on lipid peroxidation, TNF-α, IL-6, CRP, glycated hemoglobin, triglycerides, and cholesterol levels in blood of streptozotocin-treated diabetic rats. Free Radic. Biol. Med., 43 (8): 1124-31, 2007b.

51- SNYDER C.A., UDASIN I., WATERMAN S.J. and GOCHFELD M.: Reduced IL-6 levels among individuals in Hudson county, New Jersey, an area contaminated with chromium. Arch. Environ. Health., 51: 26-8,1996.

52- TAIRA M., SASAKI M., KIMURA Sh. and ARAKI Y.: Dose-dependent effects of Ni (II) ions on production of three inflammatory cytokines (TNF-α, IL-1b and IL-6), superoxide dismutase (SOD) and free radical NO by murine macrophage-like RAW264 cells with or without LPS-stimulation. J. Mater. Sci.: Mater. Med., 19: 2173-8, 2008.

53- HARKIN A., HYNES, M.J., MASTERSON E., KELLY J.P., O'DONNELL J.M. and CONNOR T.J.: A toxicokinetic study of nickel-induced immunosuppression in rats. Immunopharmacol. Immunotoxicol., 25 (4): 655-70, 2003.

54- PASCAL L.E. and TESSIER D.M.: Cytotoxicity of chromium and manganese to lung epithelial cells in vitro Toxicol. Lett., 147: 143-51, 2004.

55- COSTA M. and KLEIN C.B.: Toxicity and carcinogenicity of chromium compounds in humans. Crit. Rev. Toxicol., 36: 155-63, 2006.

56- DEWHIRST M., CAO Y. and MOELLER B.: Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. Nat. Rev. Cancer, 8: 425-37, 2008.

57- LEONARD S.S., HARRIS G.K. and SHI X.: Metal-induced oxidative stress and signal transduction. Free Rad. Biol. Med., 37: 1921-42, 2004.

58- MATES J.M., SEGURA J.A., ALONSO F.J. and MARQUEZ J.: Intracellular redox status and oxidative stress: Implications for cell proliferative, apoptosis, and carcinogenesis. Arch. Toxicol., 82: 273-99, 2008.

59- STEPNIEWSKI M., KOLARZYK E., PIETRZYCKA A., KITLINSKI M., HELBIN J. and BRZYSZCZAN K.: Antioxidant enzymes and pulmonary function in steel mill welders. Int. J. Occup. Med. Environ. Health, 6 (1): 41-7, 2003a.

60- YERER M.B., AYDOGAN S., YAPISLAR H., YALCIN O., KURU O. and BASKURT O.K.: Melatonin increases glutathione peroxidase activity and deformability of erythrocytes in septic rats. J. Pineal. Res., 35: 138-9, 2003.

61- GOULARD M., BATOREU M.C., RODRIGUES A.S., LAIRES A. and RUEFF J.: Lipoperoxidation products and thiol antioxidants in chromium exposed workers. Mutag., 20 (5): 311-5, 2005.

62- KASPRZAK K.S., SUNDERMAN F.W. JR. and SALNIKOW K.: Nickel carcinogenesis. Mutation Res., 533: 67-97, 2003.

63- KAWANISHI S., OIKAWA S., INOUE S. and NISHINO K.: Distinct mechanisms of oxidative damage induced by carcinogenic nickel subsulfide and nickel oxides. Environ. Health Perspect., 110 (5): 789-91, 2002.

64- CHEN C.Y., SHEU J.Y. and LIN T.H.: Oxidative effects of nickel on bone marrow and blood of rats. J. Toxicol. Environ. Health A., 58: 475-83, 1999.

65- CHEN C.Y., LIN T.K., CHANG Y.C., WANG Y.F., SHYU H.W., LIN K.H. and CHOU M.C.: Nickel(II)-induced oxidative stress, apoptosis, G2/M arrest, and genotoxicity in normal rat kidney cells. J. Toxicol. Environ. Health. Part A., 73 (8): 529-39, 2010.

66- KLEIN C.B., FRENKEL K. and COSTA M.: The role of oxidative processes in metal carcinogenesis. Chem. Res. Toxicol., 4: 592-604, 1991.

67- IMAMOGLU N., YERER M.B., DONMEZ-ALTUNTAS H. and SARAYMEN R.: Erythrocyte antioxidant enzyme activities and lipid peroxidation in the erythrocyte membrane of stainless-steel welders exposed to welding fumes and gases. Int. J. Hyg. Environ. Health, 211: 63-8, 2008.

68- EL SAMRA G.H., HUSSEIN A.A., FARAHAT S.A. and SAMIR A.M.: Lipid peroxidation due to trivalent chromium among tannery workers. Egypt. J. Occup. Med., 31 (1): 87-105, 2007.

69- BAGCHI D., STOHS S.J., DOWNS B.W., BAGCHI M. and PREUSS H.G.: Cytotoxicity and oxidative mechanisms of different forms of chromium. Toxicology, 180: 5-22, 2002.

70- KALAHASTHI R.B., RAO R.H., MURTHY R.B. and KUMAR M.K.: Effect of chromium (VI) on the status of plasma lipid peroxidation and erythrocyte antioxidant enzymes in chromium plating workers. Chemico-Biol. Inter., 164: 192-9, 2006.

71- KUO H.W., CHANG S.F., WU K.Y. and WU F.Y.: Chromium (VI) induced oxidative damage to DNA: Increase of urinary 8-hydroxydeoxyguanosine concentrations (8-OHdG) among electroplating workers. Occup. Environ. Med., 60 (8): 590-4, 2003.

72- CHEN C.Y., WANG Y.F., HUANG W.R. and HUANG Y.T.: Nickel-induced oxidative stress and effect of antioxidants in human lymphocytes. Arch. Toxicol., 77 (3): 123-30, 2003.

73- CHEN C.Y., SU Y.J., WU P.F. and SHYU M.M.: Nickel-induced plasma lipid peroxidation and effect of antioxidants in human blood: Involvement hydroxyl radical formation and depletion of α-tocopherol. J. Toxicol. Environ. Health A., 65: 843-52, 2002.

74- ASATIANI N., ABULADZE M., KARTVELISHVILI T., KULIKOVA N., ASANISHVILI L., HOLMAN H.Y. and SAPOJNIKOVA N.: Response of antioxidant defense system to chromium (VI)-induced cytotoxicity in human diploid cells. Biometals, 23 (1): 161-72, 2010.

75- DE MATTIA G., BRAVI M.C., LAURENTI O., DE LUCA O., PALMERI A., SABATUCCI A., MENDICO G. and GHISELLI A.: Impairment of cell and plasma redox state in subjects professionally exposed to chromium. Am. J. Ind. Med., 46: 120-5, 2004.

76- STEPNIEWSKI M., KOLARZYK E., ZAGRODZKI P., SOLARSKA K., PIETRZYCKA A., KITLINSKI M., SCHLEGEL-ZAWADZKA M. and BATKO B.: Pattern recognition methods in evaluation of the structure of the laboratory data; biominerals, antioxidant enzymes, selected biochemical parameters, and pulmonary function of welders. Biol. Trace. Elem. Res., 93 (1-3): 39-46, 2003b.

77- QUIEVRYN G., GOULART M., MESSER J. and ZHIT-KOVICH A.: Reduction of Cr (VI) by cysteine: Significance in human lymphocytes and formation of DNA damage in reactions with variable reduction rates. Mol. Cell Biochem., 222: 107-18, 2001.

78- DAS GUPTA A., DHARA P.C. and DHUNDASI S.A.: Effect of garlic (Allium sativum) on nickel II or chromium VI induced alterations of glucose homeostasis and hepatic antioxidant status under sub-chronic exposure conditions. J. Basic Clin. Physiol. Pharmacol., 20 (1): 1-14, 2009.

79- CURTIS A., MORTON J., BALAFA C., MACNEIL S., GAWKRODGER D.J., WARREN N.D. and EVANS G.S.: The effects of nickel and chromium on human keratinocytes: Differences in viability, cell associated metal and IL-1 a release. Toxicol. in Vitro, 21: 809-19, 2007.