Biomarkers, May–June 2006; 11(3): 241–249

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Plasma lipid peroxidation and erythrocyte antioxidants status in workers exposed to nickel

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Abstract

The objectives were to investigate the plasma lipid peroxidation and erythrocyte antioxidants status in workers exposed to nickel. The study groups comprised 69 nickel plating workers and 50 office workers residing in the same city, but away from the place of work of the study group subjects, considered as control group. Urinary nickel concentration was determined by graphite furnace atomic absorption spectrophotometry. The plasma lipid peroxidation and erythrocyte antioxidants were measured by spectrophotmetric methods. The plasma lipid peroxidation level was significantly increased in nickel-platers and their helpers as compared with controls. Erythrocyte antioxidants were significantly decreased in the nickel-platers compared with the controls. The level of plasma lipid peroxidation was positively and erythrocyte antioxidants were negatively and significantly correlated with the urine nickel levels. Multiple regression analysis assessed the oxidative stress associated with nickel and other potential confounding factors such as body mass index, the consumption of green vegetables, coffee, tea, smoking and alcohol consumption. Analysis showed that the lifestyle confounding factors: the consumption of green vegetables, smoking and alcohol, were not significantly associated with oxidative stress. The exposure to nickel, body mass index and coffee consumption were significantly associated with oxidative stress. The results show that the increased plasma lipid peroxidation and decreased erythrocyte antioxidants levels observed in nickel-exposed workers could be used as biomarkers of oxidative stress.

Keywords: *Urine nickel, plasma lipid peroxidation, erythrocyte antioxidants, nickel-platers*

(Received 13 July 2005; accepted 13 February 2006)

Introduction

Electroplating is the process of oxidation of metal articles by the use of electrolyte-containing acids or bases. The process of electroplating involves three steps: cleaning, plating and the post-treatment of articles. Nickel (Ni) is used as soluble salts (Ni sulphate and Ni chloride) in electroplating different articles used in a watch manufacturing process. The temperature of 50°C is maintained in the electroplating bath. At this temperature, nickel salts are decomposed into metal ions. In the biological system, nickel forms a complex with adenosine triphosphate, amino acids,

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ISSN 1354-750X print/ISSN 1366-5804 online © 2006 Taylor & Francis
DOI: 10.1080/13547500600633897

peptides, proteins and deoxyribonucleic acid (WHO Regional Office for Europe 2000). The workers engaged in this process are exposed to nickel through inhalation, ingestion and dermal contact. Inhalation is the primary route of occupational exposure to metals (Kelleher et al. 2000). The exposure to nickel causes depletion of glutathione and protein-bound sulfhydryl groups resulting in the production of reactive oxygen species (ROS). An uncontrolled production of ROS causes lipid peroxidation and DNA damage (Stohs and Bagchi 1995, Valko et al. 2005). The toxicity of nickel compounds on the production of lipid peroxidation and the decrease of antioxidants in animals has been well documented (Gupta and Shukla 1997, Ahmed et al. 1999). The studies conducted on the *in vitro* effect of nickel on human platelets, plasma and lymphocytes showed enhanced oxidative stress (Chen and Lin 2001, Chen et al. 2002, 2003). No reports are available regarding occupational exposure to nickel and the effects on oxidative stress. Therefore, the present study was undertaken to investigate the generation of free radicals involved in plasma lipid peroxidation and the potential effects on erythrocyte superoxide dismutase and glutathione peroxidase in workers exposed to nickel during the plating process.

Materials and methods

The study group involved 119 male workers who were divided into two subgroups. The first subgroup consisted of 69 workers who were employed in the nickel-plating industry located at Bangalore, India, and was considered as the nickel-exposed workers. These 69 workers were further categorized into two groups according to job categories in the industry: (1) 50 nickel-exposed workers were 'nickel-platers' who were involved in the nickel plating process and (2) 19 workers were 'nickel helpers' who were involved in loading and unloading of items and assisting the nickel-platers. The nickel-exposed workers had an exposure to nickel ranging from 10 to 20 years. The second group comprising of 50 office workers with no exposure to nickel was considered as the control group. The control group subjects were matched regarding age and socio-economic status of nickel-exposed workers.

Demographic information, work history and habits of all subjects were collected by questionnaire. The subjects with a history of diabetes or hypertension were excluded from the study.

Urine samples from the workers were collected (at the end of a shift) in metal-free polyethylene bottles and used for the estimation of nickel according to Andersen et al. (1978). The digested samples were measured for nickel in a flameless atomic absorption spectrophotometer (GBC-AAS with GF-3000). The standardization of nickel was performed with the working standard solutions of 0–30 $\mu g\ l^{-1}$. The calibration curve was linear. The internal standard of nickel 3 $\mu g\ l^{-1}$ was added to the urine and analysed, and it was found that the recovery rate was 98%. The urinary nickel was standardized with urinary creatinine concentration measured by the Jaffe reaction method developed by Husdan and Rapoport (1969).

A total of 5 ml whole blood was collected in heparinzed test tubes. Blood samples were centrifuged (3000 rpm; 10 min, 4°C) and after separation of the plasma and red blood cells, the plasma was used for the estimation of lipid peroxidation. Erythrocytes were used for the estimation of antioxidants. The concentration of the plasma lipid peroxidation was assessed through thiobarbituric acid-reactive substances by the method of Keisatoh (1978). Superoxide dismutase (SOD) activity was determined by

using a kit (Ransod; Randox Labs, Crumlin, UK, Cat No. SD 125) based on the method developed by McCord and Fridovich (1969). Glutathione peroxidase activity (GSH-Px) was performed by using a kit (Ransel; Randox Labs, Cat No. Rs 504) based on the method developed by Pagilia and Valentine (1967). The results of both the enzyme activities were expressed as units per g of haemoglobin (U g^{-1} Hb). The haemoglobin concentration was measured by using the method developed by Drabkin and Austin (1932).

Statistical analysis

SPSS package, v.7.5 for Windows, was used for the statistical analysis of the data. The χ^2-test was used to compare the demographic information among the three groups. A Student's *t*-test was used to compare means for age, body mass index (BMI) and duration of work among the groups. Pearson's correlation coefficient was used to find out the correlation between urine nickel and plasma lipid peroxidation and antioxidants. ANOVA was used to compare the oxidative stress among variables. Stepwise multiple regression analysis was used to assess the effect of the variables on oxidative stress.

Results

Table I shows the demographic details of the nickel-exposed workers: nickel-platers, helpers and control group. The average age and BMI of nickel-platers, helpers and

Table I. Demographic data for the nickel-exposed workers and control group.

Variables	Nickel-plater ($n=50$), *n* (%)	Helper ($n=19$), *n* (%)	Control group ($n=50$), *n* (%)
Age (years)	42.8±3.01[a]	42.9±3.10	42.0±4.08
BMI (kg m^{-2})	23.6±2.17	23.8±1.88	24.0±2.05
Work duration (years)	13.6±2.25	13.7±1.60	0
Consumption of green vegetables (g day^{-1}):			
Beans	20[b] (40.0)	6 (31.6)	12 (24.0)
Lady finger	10 (20.0)	4 (21.1)	12 (24.0)
Green leafs	11 (22.0)	3 (15.8)	14 (28.0)
Cabbage	9 (18.0)	6 (31.6)	12 (24.0)
Consumption of coffee and tea (cups day^{-1}):			
Tea	18 (34.0)	11 (57.9)	30 (60.0)
Coffee	26 (52.0)	7 (36.8)	15 (30.0)
None	6 (12.0)	1 (05.3)	5 (10 0)
Smoking (number of cigarettes smoked day^{-1}):			
No	45 (90.0)	17 (89.5)	45 (90.0)
Yes	5 (10.0)	2 (10.5)	5 (10.0)
Alcohol consumption (drinks $week^{-1}$):			
Usually	12 (24.0)	3 (15.8)	11 (22.0)
Sometimes	6 (12.0)	3 (15.8)	12 (24.0)
Never	32 (64.0)	13 (68.4)	27 (54.0)

[a]Mean and standard deviation.
[b]Number of persons.
Figures in parentheses are percentages.

control groups were suitably matched. The frequency distribution of life-style confounding factors such as the consumption of green vegetables, coffee, tea, alcohol consumption and smoking showed that there were no significant differences between nickel-exposed workers and control group.

Table II shows the results of univariate analysis of variables that affect the concentrations of plasma lipid peroxidation and antioxidants. The variables were as follows: exposure and non-exposure to nickel, correlation between urine nickel and plasma lipid peroxidation and antioxidants, consumption of green vegetables, BMI, consumption of coffee and tea, alcohol consumption and smoking. The level of

Table II. Univariate analysis of variables that affect the plasma lipid peroxidation and antioxidants ($n = 119$).

Variables	n	Plasma lipid peroxidation (nmol ml^{-1})[d]	Superoxide dismutase (U g^{-1} Hb)[c]	Glutathione peroxidase (U g^{-1} Hb)[c]
Group:				
Nickel-platers	50	3.54 ± 0.93**[a]	1188 ± 44**	49.2 ± 6.79**
Helpers	19	3.27 ± 0.98*	1212 ± 41	52.0 ± 5.72
Control	50	2.63 ± 0.68	1206 ± 38	53.9 ± 7.07
Correlation with urine nickel:				
Nickel-platers	50	0.546**[b]	−0.725**	−0.829**
Helpers	19	0.525*	−0.633**	−0.612**
Control	50	0.024	0.054	0.024
Consumption of green vegetables (g day^{-1}):				
Beans	38	3.16 ± 0.90	1204 ± 38	52.0 ± 7.15
Lady finger	26	3.22 ± 1.16	1204 ± 47	51.7 ± 7.68
Green leaves	28	2.94 ± 0.79	1188 ± 52	51.0 ± 7.34
Cabbage	27	2.97 ± 0.83	1199 ± 29	52.2 ± 6.19
Body mass index (kg m^{-2}):				
≤25	90	3.10 ± 0.93	1199 ± 41	51.0 ± 7.00
>25	29	2.97 ± 0.91	1201 ± 44	53.0 ± 7.09
Consumption of coffee or tea (cups day^{-1}):				
Tea	59	3.01 ± 1.00	1198 ± 40	52.6 ± 6.74
Coffee	48	3.16 ± 0.80	1196 ± 39	51.0 ± 6.20
None	12	2.94 ± 1.08	1214 ± 61	51.1 ± 10.9
Smoking (number of Cigarettes smoked day^{-1}):				
Yes	12	3.08 ± 0.89	1199 ± 41	51.8 ± 5.36
No	107	3.08 ± 1.22	1202 ± 53	51.6 ± 7.23
Alcohol consumption (drink week^{-1}):				
Usually	26	3.38 ± 0.92	1198 ± 47	52.6 ± 7.78
Some times	21	3.10 ± 0.93	1198 ± 53	52.0 ± 7.95
Never	72	2.96 ± 0.91	1200 ± 36	51.3 ± 6.60
Urine nickel (μg g^{-1} creatinine):				
Low (<5.0)	42	2.70 ± 0.75	1221 ± 43	55.8 ± 6.39
Moderate (6–10)	50	2.94 ± 0.82	1200 ± 29**	52.0 ± 5.02**
High (>11)	27	3.91 ± 0.84**	1162 ± 36**	45.0 ± 6.26**

[a]Mean and standard deviation.
[b]Correlation coefficient (r).
[c]Units g^{-1} haemoglobin.
[d]Nano moles of lipid peroxide ml^{-1} plasma.
*Significant at $p < 0.05$ and **$p < 0.01$.

plasma lipid peroxidation was significantly increased in the nickel-exposed workers (nickel-platers and helpers) as compared with the controls. The levels of antioxidants: superoxide dismutase and glutathione peroxidase, were significantly decreased in nickel-platers compared with the control group, and no significant difference was observed among helpers with respect to antioxidant levels. The plasma lipid peroxidation was significantly ($p < 0.01$) higher in subjects who had high urinary nickel levels and the levels of antioxidants were significantly ($p < 0.01$) lower in subjects who had moderate and high urinary nickel levels. However, there were no significant differences observed for confounding factors such as the consumption of green vegetables, BMI, consumption of coffee and tea, smoking and alcohol consumption.

A positive correlation was found between urine nickel and plasma lipid peroxidation in nickel-exposed workers and control group. The correlation coefficient (r) was 0.546 in nickel-platers, 0.525 in helpers and 0.024 in the control group. The correlation coefficient among nickel-platers were significant at $p < 0.01$; in helpers it was $p < 0.05$. The correlation coefficient was not significant in the control group.

A negative correlation was found between urine nickel and antioxidants: superoxide dismutase and glutathione peroxidase in the nickel-exposed workers as compared with the controls. The correlation coefficient (r) between urine nickel and superoxide dismutase was -0.725 in nickel-platers, -0.633 in helpers and 0.054 in controls. The correlation coefficient (r) between urine nickel and glutathione peroxidase was -0.829 in nickel-platers, -0.612 in helpers and 0.024 in the control group. The correlation coefficient between urine nickel and antioxidants among the nickel-platers and helpers were significant at $p < 0.01$. The correlation coefficient between urine nickel and antioxidants in the control group was not significant.

Table III shows the results of stepwise multiple regression analysis of variables that affect the concentrations of plasma lipid peroxidation and antioxidants. The variables, included in the regression model, were different job categories (1 = nickel-platers, 2 = helpers, 3 = control group), BMI (1 = $\leq$25, 2 = $>$25 kg m^{-2}), the consumption of green vegetables, coffee and tea (1 = coffee, 2 = tea, 3 = none), smoking (0 = no, 1 = yes), alcohol consumption (usually = 1, sometimes = 2, never = 3) and the levels of urine nickel (three groups: $<$5, 6 $-$ 10 and $>$11 μg g^{-1} creatinine). Multiple regression analysis showed that in both nickel-platers and helpers there was considerable influence by exposure of nickel on plasma lipid peroxidation, superoxide dismutase and glutathione peroxidase. However, the association was highly (72%) significant for all three parameters in nickel-platers. However, in helpers such correlation was noticed (74%) as with glutathione peroxidase levels. In control group, the nickel had no influence on oxidative stress. It was noted that a BMI of $\leq$25 kg m^{-2} had a significant influence (61%) on the levels of plasma lipid peroxidation, superoxide dismutase and glutathione peroxidase, whereas in people with BMI of $>$25 kg m^{-2} such an association (39%) was noted only with plasma lipid peroxidation. The consumption of coffee influences on plasma lipid peroxidation and superoxide dismutase and it explains 49%. The urine nickel levels more than 11 μg g^{-1} creatinine appeared to have an influence (49%) on glutathione peroxidase. The other confounding factors such as the consumption of green vegetables, tea, alcohol and urine nickel less than 5 and 6–10 μg g^{-1} creatinine did not show any significant influence on the status of plasma lipid peroxidation and erythrocyte antioxidants.

Table III. Multiple regression analysis of variables affect the plasma lipid peroxidation and antioxidants ($n = 119$).

Variables	Plasma lipid peroxidation (nmol ml^{-1}), β (p-value)	Superoxide dismutase (U g^{-1} Hb)[a], β (p-value)	Glutathione peroxidase (U g^{-1} Hb)[a], β (p-value)	R^2
Nickel-platers	1.86 (0.004)*[b]	−0.046 (0.004)*	−0.533 (0.000)*	0.72
Helpers	0.05 (0.650)c	−0.029 (0.085)	−0.305 (0.015)*	0.74
BMI (kg m^{-2}):				
≤25	2.118 (0.000)*	−0.003 (0.010)*	−0.266 (0.000)*	0.61
>25	1.899 (0.000)*	−0.263 (0.083)	−0.059 (0.714)	0.39
Consumption of coffee or tea:				
Coffee = 1	2.363 (0.000)*	−0.095 (0.402)	−0.251 (0.000)*	0.49
None = 3	1.746 (0.010)*	−0.005 (0.000)*	−0.259 (0. 059)	0.51
Smoking (0 = no)	2.244 (0.000)*	−0.142 (0.100)	−0.325 (0.000)*	0.54
Alcohol consumption (never = 3)	1.981 (0.000)*	−0.003 (0.036)*	−0.261 (0.002)*	0.60
Urine nickel ≥11	0.127 (0.428)	−0.302 (0.075)	−0.521 (0.000)*	0.49

β (p-values) = regression coefficient (p-values of regression coefficients).
[a]Units g^{-1} haemoglobin.
[b]Regression coefficient and p-value. *Significant at $p < 0.05$.
[c]Regression coefficient and p-value indicated in brackets without a mark is not-significant.
U = 1 unit superoxide dismutase enzyme defined as the inverse amount of required for 50% inhibition.
U = 1 unit of glutathione peroxidase enzyme activity was defined as μmol NADPH oxidized min.

Discussion

The study assessed the status of plasma lipid peroxidation and antioxidants in workers exposed to nickel during the nickel-plating process. The absorption of nickel is quantified in the urine samples of nickel-exposed and non-exposed workers. In the current study, the urine nickel levels of nickel-exposed workers were significantly higher than in the control group. The level of urine nickel was similar to that published by Sunderman (1993). The level of nickel in urine was considered an indicator of the bioavailblity of nickel (Sunderman et al. 1988). During the present study, it was noted that plasma lipid peroxidation was significantly increased and the levels of antioxidants were significantly decreased in nickel-platers than in the control group. In the case of helpers, the level of plasma lipid peroxidation was significantly increased as compared with the control group. However, the levels of antioxidants were not significant. The level of plasma lipid peroxidation was positively and antioxidants were negatively and significantly correlated with levels of nickel in the urine in nickel-platers and helpers. Since the oxidative stress is related to life-style confounding factors such as BMI, the consumption of green vegetables, coffee, tea, smoking and alcohol consumption, the present study was carried to assess the correlation between the oxidative stress and the life-style confounding factors.

Ozata et al. (2002) and Ohmori et al. (2005) reported an association between BMI and the levels of plasma lipid peroxidation, superoxide dismutase and glutathione peroxidase. During the present study, univariate analysis did not indicate any such association. However, multiple regression analysis indicated that BMI significantly influenced the plasma lipid peroxidation, superoxide dismutase and glutathione peroxidase levels.

Riso et al. (2004) and Sanchez-Moreno et al. (2004) reported that there is a reduction of oxidative stress in subjects who consumed vegetables. The present study did not find any such association in both univariate and multiple regression analysis.

Mursu et al. (2005) found that the long- or short-term consumption of coffee did not have any detectable effect on lipid peroxidation and the antioxidant enzymes level in healthy non-smoking men. The present study showed that the consumption of coffee had a significant effect on the plasma lipid peroxidation and glutathione peroxidase in multiple regression analysis, but not in univariate analysis. This apparent contradiction may explain the fact that there was a strong intercorrelation between coffee consumption and smoking. Rietveld and Wiseman (2003) reported that tea, which contains high levels of flavonoids, can protect the cells and tissues from oxidative damage by scavenging oxygen-free radicals. The results of the present study suggest that consumption of tea has no effect on oxidative stress.

Nalini et al. (1999) reported oxidative stress in patients with alcoholic cirrhosis. Lecomte et al. (1994) reported higher levels of plasma lipid peroxidation in chronic alcoholics than in the groups with low and moderate alcohol consumption. During the present study, it was noted that workers who consumed alcohol occasionally had an increased level of plasma lipid peroxidation and a decreased level of super oxide dismutase. On evaluation, it was noted that it was not significant, since the majority of these workers consumed alcohol in moderate or low amounts. The univariate and multiple regression analysis showed that the consumption of alcohol did not influence oxidative stress.

Koylu et al. (2000) reported a significant increase in plasma lipid peroxidation, superoxide dismutase and glutathione peroxidase among teenage smokers. Nielsen et al. (1997) reported increased plasma lipid peroxidation in daily smokers. The present study noted increased levels of glutathione peroxidase in smokers when compared with levels in non-smokers, but it was not significant, since the smokers in the present study were occasional smokers. Smoking also does not have any impact on oxidative stress. The increased lipid peroxidation and decreased antioxidants were associated with nickel exposure and lifestyle factors such as BMI and coffee consumption.

In conclusion, the plasma lipid peroxidation and antioxidant status among nickel-exposed workers were significantly altered in the nickel-exposed workers as compared with the levels in the control group. The level of plasma lipid peroxidation was positively and significantly correlated with urine nickel levels. The levels of antioxidants in the nickel-platers were negatively and significantly correlated with urine nickel levels. The increased plasma lipid peroxidation and decreased antioxidants status among nickel-exposed workers were considered as biomarkers of oxidative stress. Hence, these tests could be used as biomarkers of oxidative stress in nickel-exposed workers to prevent further damage due to oxidative stress.

Acknowledgements

The authors are grateful to the Director, National Institute of Occupational Health, Ahmedabad, for his encouragement and support throughout the study. They are thankful to Mrs A. Mala for technical assistance. They acknowledge the help received from Dr Subbukrishna, Professor of Biostatistics, National Institute of Mental Health and Neurosciences, Bangalore, during the statistical evaluation of the data.

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